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LC-MS/MS Quantitative Method for Trace Analysis of Pesticides in Urban/Suburban Water Wells in Milwaukee, Ozaukee, Washington, and Waukesha Counties in Wisconsin

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LC-MS/MS QUANTITATIVE METHOD FOR TRACE ANALYSIS OF PESTICIDES IN URBAN/SUBURBAN WATER WELLS IN MILWAUKEE, OZAUKEE, WASHINGTON, AND WAUKESHA COUNTIES IN WISCONSIN

by

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ABSTRACT

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Under the Supervision of Professor Yin Wang, PhD

The aim of this study was to develop a LC-MS/MS analytical method to quantitate a selection of pesticides that included: 2,4−dichlorophenoxyacetic Acid (2,4-D), carbaryl, dicamba, imidacloprid, malathion, 2-methyl-4-chlorophenoxyacetic acid (MCPA), and methylchlorophenoxypropionic acid (MCPP). Chromatographic and mass spectrum conditions were optimized by analyzing full scans, selected ion monitoring (SIM) scans, product ion (PI) scans, which developed a multiple reaction monitoring (MRM) scan. Additionally, a pre-treatment and cleanup strategy was designed and optimized using liquid-liquid extraction. The method demonstrated acceptable mean percent recovery of 83.2% ±12.56% at a spiked level of 10 ng/mL. The developed quantitative method was applied to groundwater from 16 active, private wells located in the Milwaukee metropolitan area. There were eight wells that detected one or more of the targeted pesticides during four sampling events. Seven out of the eight pesticides were detected in June-July and August 2019.

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2,4-D	2,4-Dichlorophenoxyacetic Acid
ACN	Acetonitrile
AH	Ammonium Hydroxide
APCI	Atmospheric Pressure Chemical Ionization
CE	Collision Energy
DCM	Dichloromethane
ES	Enforcement Standard
ESI	Electrospray Ionization
FA	Formic Acid
GC	Gas Chromatography
HCl	Hydrochloric Acid
LC	Liquid Chromatography
LC-MS	Liquid Chromatograph-Single Quadrupole Mass Spectrometer
LC-MS/MS	Liquid Chromatograph-Tandem Mass Spectrometer
LOQ	Limit of quantitation
LLE	Liquid-Liquid Extraction
MCPA	2-Methyl-4-Chlorophenoxyacetic Acid
MCPP	2-(4-Chloro-2-methylphenoxy)propanoic acid
MeOH	Methanol
MS	Mass spectrometry
MRM	Multiple Reactions Monitoring
m/z	Mass-to-Charge
PI	Product Ion
SPE	Solid Phase Extraction
SIM	Selected Ion Monitoring

CHAPTER 1: INTRODUCTION

1.1 Background

Pesticides are a class of substances that control the growth of weeds, fungi and insects in both agriculture and residential settings. Agriculture land is estimated to use 68% of the total pesticide produced in the United States (Zhang et al., 1997). Meanwhile, residential use, for home and garden, primary in urban and suburban areas, accounts for approximately 10% of total pesticide use in the United States (Atwood et al., 2017). U.S. Environmental Protection Agency (U.S EPA) authorizes over 200 different pesticides to be used for residential purposes. Of the 200 different pesticides, there are 30 that are most commonly applied. A survey conducted by the Environmental & Human Health, Inc. (EHHI) states that approximately 75% of homeowners use pesticides on their lawns (EHHI, 2003). The EHHI also mentions that homeowners are applying up to 10 times more pesticides than farmers use on their crops per acre (EHHI, 2003). In 2012, it was approximated that more than 50 million pounds of active ingredients of home and garden pesticides (herbicide, insecticide, fungicide) were used in the United States (Atwood et al., 2017). It is estimated that less than 0.1% of the pesticide applied reaches the intended target pest (Arias-Estevez et al., 2008). Consequently, pesticide that enter the environment have the potential to contaminate soil, water, and air, which can negatively affect organisms they were not intended for.

When applied, some active ingredients can be transported via a range of different pathways which can percolate into groundwater and surface water (Pullan, 2016). The use of pesticides for agriculture and residential purpose has had a significant impact on groundwater quality in the United States. Presently, the occurrence of pesticides in groundwater has been

monitored with emphasis on agriculture land by government agencies such as Department of Agriculture Trade and Consumer Protection (DATCP), Department of Natural Resources (DNR), U.S Geological Survey (USGS) and others. Results from the USGS monitoring program indicate that 97% of surface water samples from both agricultural and (sub)urban areas contain one or more pesticides at detectable levels (Gilliom, 2007). Meanwhile, it is estimated that in the United States at least 46 different agricultural pesticides have been found in groundwater samples in 26 states (Zhang et al., 1997). In urban and suburban areas, 55% of the shallow groundwater samples have shown detectable pesticide levels (Gilliom, 2007). In some cases, the water sources that the pesticides are transported to are used as a drinking water source.

In a study conducted by the USGS in 2007, pesticides were less common in groundwater than in streams, but occurred in more than 50% of wells that sampled shallow groundwater beneath agricultural and urban areas (Gilliom, 2007). However, despite the routine investigation and monitoring programs for groundwater contamination by agricultural pesticides, limited information is available on the occurrence and levels of home and garden pesticides in groundwater in non-agricultural land (e.g., residential areas), and the associated risk of their exposure to residents.

Considering the common overuse of home and garden pesticide, and the widespread presence of private drinking water wells in suburban areas, the lack of residential pesticide monitoring potentially poses health risks to the public. Furthermore, although groundwater is less vulnerable, it is still important to monitor since contamination is difficult to reverse.

1.3 Target Pesticides

1.3.1 Overview of Target Pesticides

The U.S. EPA authorizes over 200 different pesticides to be used for residential purposes. However, this study analyses some of the most common active ingredients in popular home and garden pesticides. In descending rank order, the 10 most used conventional active ingredient for home and garden pesticides in 2012 include 2,4-dichlorophenoxyacetic Acid (2,4-D), glyphosate, methylchlorophenoxypropionic acid (MCPP), pendimethalin, carbaryl, acephate, permethrin and other pyrethroids, dicamba, 2-methyl-4-chlorophenoxyacetic acid (MCPA), and malathion (Atwood et al., 2017). Of the previously mentioned pesticides, 2,4-D, MCPP, MCPA, dicamba, carbaryl, and malathion were selected as target pesticides for this study. In addition to the mentioned pesticides, imidacloprid was also included as a target pesticide. Figure 1 shows the chemical structure of the target pesticides that were chosen. These seven pesticides were selected because of their high-water solubility, moderate to low soil organic carbon-water

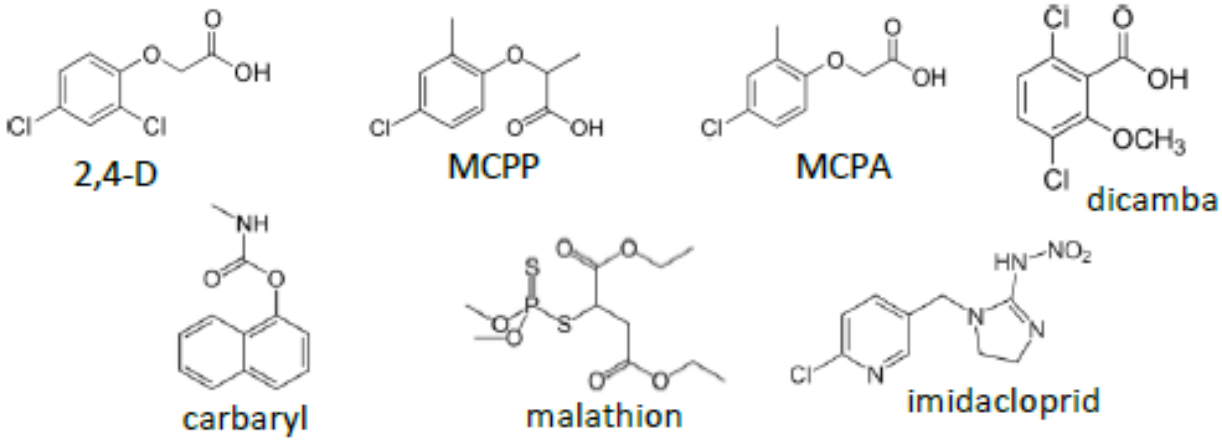

Figure 1 Home and Garden Target Pesticides

partitioning coefficient (K_{oc}) and relatively long half-life (**Error! Reference source not found.**) (PAN, 2019). Furthermore,

	Target Pesticide	Molecular Weight	Class (Type*)	Chemical Name
			Neutral/Basic	
1	Carbaryl	201.22	Carbamate (I)	1-Naphthyl Methylcarbamate
2	Imidacloprid	255.66	Neonicotinoid (I)	1-(6-Chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine
3	Malathion	330.36	Organophosphate (I)	Diethyl 2-Dimethoxyphosphinothioylsulfanylbutanedioate
			Acidic	
4	MCPA	200.62	Phenoxy Acid (H)	2-Methyl-4-Chlorophenoxyacetic Acid
5	MCPP	214.65	Phenoxy Acid (H)	2-(4-Chloro-2-methylphenoxy)propanoic acid
6	2,4-D	221.04	Phenoxy Acid (H)	2,4-Dichlorophenoxyacetic Acid
7	Dicamba	221.04	Methoxybenzoic (H)	3,6-dichloro-2-methoxybenzoic acid
*Note: I = Insecticide, H = Herbicide				

shows the chemical name, molecular weight, class of pesticide and the type of pesticide. All 7 pesticides selected have been previously observed in numerous groundwater sources across the globe, particularly in shallow groundwater (Hill et al., 1996; Buss et al., 2006; Gilliom, 2007; Newhart, 2006; Bonmatin et al., 2015).

Table 1 Physical Property Data for Target Pesticides

Pesticide	Avg. Water Solubility (mg/L)	K_{oc} (L/kg)	Avg. Hydrolysis half-life ($t_{1/2}$, d)	Avg. Aerobic soil half-life ($t_{1/2}$, d)	Avg. Anaerobic soil half-life ($t_{1/2}$, d)
Carbaryl	116	375	12	6	87
Imidacloprid	514	262	30	997	27
Malathion	125	219	6	3	30
MCPA	29390	74	N.A	15	N.A
MCPP	734	26	31	13	541
2,4-D	27,600	46	39	34	333
Dicamba	27,200	5	30	10	88
* All values are cited from PAN Pesticide Database at http://www.pesticideinfo.org/					

Table 2 Home and Garden Selected Pesticides

	Target Pesticide	Molecular Weight	Class (Type*)	Chemical Name
			Neutral/Basic	
1	Carbaryl	201.22	Carbamate (I)	1-Naphthyl Methylcarbamate
2	Imidacloprid	255.66	Neonicotinoid (I)	1-(6-Chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine
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6	2,4-D	221.04	Phenoxy Acid (H)	2,4-Dichlorophenoxyacetic Acid
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*Note: I = Insecticide, H = Herbicide				

1.3.2 Toxicity of Target Pesticides

Many studies over the past few decades have suggested that the toxicity of pesticides may pose adverse health effects to the public. Toxicity is defined as the adverse effects caused by the interference of specific agents to the structure and/or processes which are essential for survival and proliferation of an organism (Chinalia et al., 2007). Pesticides that enter the environment unnecessarily can contaminating the soil, water, and air, where they can poison or otherwise adversely affect nontarget organisms. Pesticides and their metabolites may pose adverse health effects, such as birth defects, kidney/liver damage and neurotoxicity (EHHI, 2003).

2,4-D has been used extensively in modern agriculture and studies have shown that it has a great potential for inducing unwanted effects on organisms. Depending on the organisms, concentration, and time of exposure, 2,4-D may produce a toxic effect ranging from embryo toxicity and teratogenicity to neuro-, immuno- and hepatotoxicity (Chinalia et al., 2007).

Three of the target pesticides - 2,4-D, MCPP and Dicamba - have been associated with non-Hodgkins Lymphona (NHL) in epidemiological studies (Davis et al., 1993; Hardell et al., 2002; Lynge, 1995; McDuffie et al., 2001). Additionally, an acute MCPA poisoning in humans can cause nausea, vomiting, stomachache, diarrhea, headache, dizziness, muscle fasciculation, hypotension, dyspnea, liver and kidney dysfunction (Takayasu et al., 2008).

The World Health Organization (WHO) reported on the acute toxicity of the pure chemicals. The WHO based its ratings on the lowest lethal dose that kills 50% of the tested rats. The pesticides from this study ranking are shown in Table 3 (WHO, 2010).

Table 3 WHO Acute Hazard Ranking

Pesticide	WHO Acute Hazard Ranking
Carbaryl	II, Moderately Hazardous
Imidacloprid	II, Moderately Hazardous
Malathion	III, Slightly Hazardous
MCPA	III, Slightly Hazardous
MCPP	III, Slightly Hazardous
2,4-D	II, Moderately Hazardous
Dicamba	III, Slightly Hazardous

1.3.3 Wisconsin Groundwater Standards and Health Criteria

In Wisconsin, groundwater standards protect the groundwater by limiting the number of harmful substances that can be discharged into it. The Wisconsin Department of Natural Resources Chapter 140 (WI NR 140) currently enforces groundwater quality based on a health-based enforcement standards and preventative action limits. A preventative action limit, according to WI NR 140, is the concentration that serves to inform of potential groundwater contamination problems and informs the department that it may require to begin efforts to control the contamination (WI NR 140, 2020). From this study, only 3 of the 7 pesticides currently have an enforcement standard and preventative action limit (

Table 4). Furthermore, standards for drinking water differ and can be found in the Department of Natural Resources chapter 809 (WI NR 809, 2018). WI NR 809 establishes standards as maximum contaminant level (MLC). Only one of the pesticides in this study currently has an MCL for drinking water (

Table 4).

Table 4 Wisconsin Groundwater and Drinking Water Standards

	WI NR 140.10		WI NR 809.07
Pesticide	Enforcement Standard (ES) (ng/mL)	Prevention Action Limit (ng/mL)	Maximum Contaminant Level (MCL) (ng/mL)
Carbaryl	70	4	*
Imidacloprid	*	*	*
Malathion	*	*	*
MCPA	*	*	*
MCPP	*	*	*
2,4-D	70	7	70
Dicamba	300	60	*
* no data found			

1.4 Pesticide Measurement Overview

Pesticide detection methods falls under two analysis: targeted and non-targeted analyses. This study aims to develop a method for targeted analysis which aims to detect and/or quantify a set of known chemical compounds. Measurement of target analysis steps include pre-treatment and detection. Pre-treatment method aims to separate analytes from the environmental sample and concentrate them for measurement. Detection, done by an analytical method, aims to detect, verify, and quantify the target analytes. The challenge is selecting, developing, optimizing, and validating these steps to function for all the target analytes.

1.5 Goals and Objectives

This project focus is groundwater quality in Wisconsin's southeastern (sub)urban areas. It focuses on the importance of groundwater contamination by home and garden pesticides in non-agricultural land or agri-urban area. The goal of this study was to develop an analytical method to

identify and measure 2,4-D, MCPP, MCPA, dicamba, imidacloprid, malathion, and carbaryl in a single experimental run.

As will be discussed in Section 2.2, there are several challenges regarding quantitative analysis of target pesticides in environmental samples. These include, but not limited to, trace quantitation of multiple compounds, and limitations with respect to instrument resolution, sensitivity, and accuracy.

To address these challenges and develop an analytical method that would be efficient and reliable for quantifying the target pesticides, the following objectives were met: Identify an analytical method that would be suitable for all the target pesticides simultaneously, develop and optimize the analytical method for trace analysis, validation of the developed method, and finally, application of methods to groundwater samples collected from Wisconsin's southeastern (sub)urban areas.

1.6 Organization

This thesis is divided into 5 chapters. Chapter 1 introduces the target pesticides and gives an overview of their chemical properties, toxicity, and established Wisconsin groundwater and drinking standards. Also mentioned in chapter 1 are the goals, pesticide measurement overview, and objectives of the study. Chapter 2 presents current analytical methods used to quantify the target pesticides, as well as introduces the chosen method. Chapter 3 goes into detail regarding the process and results of the chosen analytical method. This includes quantitative, optimization, and method validation of chromatographic and mass spectrometry conditions. Chapter 4 presents results from the application of developed and validated analytical method on multiple groundwater well locations in Wisconsin's southeastern (sub)urban areas. Chapter 5 summarizes the results of the study, discusses limitations, as well as provides recommendations for future works.

CHAPTER 2: PRE-TREATMENT AND ANALYTICAL METHODS FOR QUANTIFYING TARGET PESTICIDES

2.1 Introduction

Analytical methods consist of coupling a physical method and a detector to examine the target analytes. The challenge is choosing an analytical method that is suitable for all 7-target pesticides. One of the common physical methods used is chromatography.

Chromatography is the physical method of separating compounds from two phases, stationary phase and mobile phase. An incorrect stationary or mobile phase can prevent an effective analysis. There are different chromatographic techniques in use, but the most common to analyze and quantify the pesticides from this study are liquid chromatography (LC) and gas chromatography (GC). These physical separation techniques have been used to detect and quantify the target pesticides by various governmental agencies, such as U.S. EPA, USGS, and independent researchers.

The drawback of GC is that it often requires chemicals to undergo derivatization prior to analytical analysis, particularly the phenoxy acids, such as MCPP, MCPA and 2,4-D (Budde, 2004, Tran et al., 2007). Derivatization would be an additional procedure that would lengthen the total analysis time. LC, on the other hand, would not require derivatization.

In order to quantify compounds, physical separation methods are combined with a detector. The choice of detector is a crucial part to the success of a particular method. They allow for sensitive and clear identification of intended target analytes. In the case of GC, detectors commonly used are electron capture, nitrogen-phosphorous, and/or flame photometric detectors. Methods that use LC are coupled with UV, diode array and fluorescent

detectors. However, the most frequently used detector for both GC and LC is mass spectrometry (MS). MS has become a common detector in modern day analytical methods. One of the major advantages of MS is its allowability to differentiate compounds that may have similar retention times and/or similar molecular weights. It improves the sensitivity by verifying and confirming compounds by the selection of molecular weight and fragment ions (Alder et al., 2006). However, if a set of compounds have a similar fragmentation ion, which was the case in this study (section 3.4.3), an additional fragmentation is needed for further confirmation. The addition of a second MS detector to liquid chromatography mass spectrometry (LC-MS) is recognized as liquid chromatography tandem mass spectrometry (LC-MS/MS). The addition of a second MS aids in confirming target pesticides by monitoring the fragmentation of the precursor ion into product ions. LC-MS/MS has demonstrated to have the most effective analytical capability for detection and quantitation of the target pesticides because of their sensitivity, accuracy, and short analysis time (Hu et all., 1999; Rodrigues et al., 2007; Tran et al., 2007; Pitarch et al., 2016).

2.1.1 LC-MS/MS

LC-MS/MS is an analytical technique that separates individual compounds from a mixture while quantifying the amount of each individual compound present in a sample. LC-MS/MS is the combination of liquid chromatography, a physical separation technique, and tandem mass spectroscopy, a detection technique.

In liquid chromatography, a liquid sample that may contain chemicals is injected into a column. The columns material (stationary phase) retains the chemicals that are being analyzed. The column is then flushed using an eluent liquid(s) (mobile phases). The chemicals are then

separated as they move through the column based on differing physicochemical interactions between the stationary phase and mobile phases. The mobile phases are liquids that can consist of organic, aqueous or a combination of both. Furthermore, the mobile phase(s) flow characteristics can be isocratic, a continuous concentration of solvent, or a gradient, a concentration of mobile phase that changes over time. The chemicals being analyzed are eluted from the column at different rates (retention time) based on their molecular size, charge, hydrophobicity, binding interactions, or a combination of these factors. The retention time dictates how well the detector can effectively define peak shape for quantitation.

MS measures the physical characteristics of the target analyte by fragmenting them to ions (precursor ion). The precursor ions are moved and manipulated according to their mass-to-charge (m/z) value in the detector due to their magnetic deflection. The separated ions are then measured by the mass analyzer. However, precursor ions from different analytes can have the same retention time and/or m/z value. To distinguish these ions from one another, a second MS detector is used in tandem.

Tandem mass spectrometry (MS/MS) provides the ability to further fragment the precursor ions and produce a product ion (PI). Producing a PI aids in reliably confirming that the precursor ion is from the correct target analyte. Identifying a PI is critical if two or more target analytes have a similar precursor ion m/z value.

In short, LC-MS/MS is both effective and efficient, by reducing analysis time and solvent consumption and verifying via a product ion if two of the target pesticides have a similar precursor m/z value.

2.2 Preconcentration Method

Unlike samples prepared in the lab with purified water, environmental samples may contain suspended particulate matter, impurities, and low concentration levels of targeted and untargeted analytes. Although LC-MS/MS is capable of detecting low concentrations of analytes, environmental samples may have concentrations that are significantly lower than detectable. For that reason, environmental sample are generally purified and preconcentrated prior to analysis. Preconcentration sample preparation is usually the most critical step on the quantifying process. The two methods that are commonly used for preconcentration are liquid-liquid extraction (LLE) (Tran et al., 2007; Thorstensen et al., 2000) and solid phase extraction (SPE) (Robles-Molina et. al., 2014; Rodrigues et al., 2007).

SPE is designed to extract, absorb, and/or partition one or more compounds from a sample onto a stationary phase, sorbent, or resin. Then the target analytes are eluded from the stationary phase with the aid of a mobile phase. The effectiveness of SPE is based on the interaction of the target analyst with the stationary phase, sample flow rate through the stationary phase, elution mobile phase chosen and elution flow rate.

LLE is a well-established and simple preconcentration method. This method separates compounds or metal complexes based on two different unmixable liquids, usually between aqueous and organic solvents. The extraction occurs when one or more species are transferred from one solvent to the other. Once the desired analytes are transferred to the desired solvent, it is concentrated by evaporating the solvent. The effectiveness of LLE is greatly based on the interaction of the target analytes with the extracting solvent.

The major hurdle concerning preconcentration method is choosing one that is suitable for all the target analytes. The preconcentration method must have a satisfactory time frame, reproducibility and, most importantly, acceptable recovery for all the target analytes. Efforts were made to study, reproduce, and establish a SPE and LLE method that would be an effective method. Section 3.3 details the pre-treatment methods that were examined and the results presented in section 3.5.4.

2.3 Conclusion

The purpose of this study was to develop an analytical method to identify and quantify all 7 target pesticides in a single experimental run. After reviewing different analytical techniques, it was concluded that the use of LC-MS/MS was the best option for quantifying the target analytes. However, some challenges still needed to be addressed. These challenges included, but not limited to, trace quantitation of multiple compounds, limitations with respect to instrument resolution, sensitivity, and linearity.

To address these challenges and develop an effective and reliable analytical method the following objective were met: development and optimization of an efficient LC-MS/MS quantitative analytical method for trace analysis of all 7 pesticides (section 3.2), validation of the developed quantitative method (section 3.3), and application of the method to groundwater well samples collected within Milwaukee metropolitan area (chapter 4).

CHAPTER 3: PESTICIDE MEASUREMENT METHOD DEVELOPMENT

3.1 Introduction

LC–MS/MS methods generally have shorter analysis times with improved linearity and sensitivity compared to other quantitative methods. Although there are existing instrument parameters for the target analytes using LC-MS/MS, they do not apply to all the target analytes of this study in one experimental run. Therefore, this study focuses on developing and optimizing parameters that can analyze all the target analytes simultaneously. In order to develop an effective analytical method for the LC-MS/MS, chromatographic and mass spectroscopy conditions were optimized.

Chromatographic conditions examined included: mobile phase, stationary phase, column temperature, injection volume and flow rate. Each of the parameters was optimized to produce adequate retention times and peak definitions for each analyte.

The four types of MS/MS scans that were examined included: a full scan, selected ion monitoring (SIM) scan, product ion (PI) scan, and multiple reactions monitoring (MRM) scan. Additionally, since the chemical compounds ionize at different rates, the ion source and source needle position were also evaluated. The process and results for chromatographic conditions, mass spectroscopy conditions are discussed in the following section. After the method optimization, the method reliability was examined.

After the LC-MS/MS was established, an effort was made to create a pre-treatment process in order to separate and concentrate the target analytes from a large sample volume. The pre-treatment processes that were investigated were SPE and LLE. SPE conditions examined were sorbent median and elution solvent. LLE processes examined included choosing

a solvent that would be effective for separating the target analytes from the water sample. The solvents used, diethyl ether and dichloromethane (DCM), were selected based on previously published works (Tran et al., 2007, Thorstensen et al., 2000) and established methods (U.S. EPA Method 8151A, U.S. EPA Method 651, and USGS Method 5-C3). Additionally, as part of the final step in SPE and LLE processes, the samples undergo nitrogen evaporation. To confirm that there was minimal loss during the evaporation step, the evaporation procedure was also analyzed.

Method validation is critical to the success of the study. Method validation is the process of proving that an analytical method is acceptable for its intended purpose (Green, 1996). Method validation includes, but not limited to, specificity, selectivity, linearity, accuracy, and limit of quantitation.

3.2 LC-MS/MS Method

3.2.1 Chemicals, Reagents, and others

Seven pesticides and two surrogate standards were used in the study. The pesticides include 2,4-D, dicamba, MCPP, MCPA, imidacloprid, malathion, and carbaryl which were purchased from Sigma-Aldrich (St Louis, MO, USA). The two-surrogate standards which included MCPA-13C6 (RING-13C6, 99% purity) and imidacloprid-D4 (4,4,5,5-D4, 98% purity) were purchased from Cambridge Isotope Laboratories, Inc (Andover, MA, USA).

Acetonitrile (HPLC Grade), dichloromethane (HPLC grade), diethyl ether (ACS Grade), and hydrochloric acid (36.5-38%, ACS Grade) were purchased from VWR Chemicals (Radnor, PA, USA). Whatman® glass microfiber Filter (Grade GF/B: 1.0 µm, Diameter 47mm) was purchased from Sigma Aldrich. Sulfuric acid (96%, ACS grade) was purchased from Fisher Science

(Hampton, NH, USA). Analytical UHPLC column (Kinetex® 1.7 µm EVO C18 100 Å 100 x 2.1 mm) was purchased from Phenomenex.

3.2.2 Standard preparation

Primary stock solution for each target analyte was prepared separately in acetonitrile at a concentration of 100 µg/ml. The primary surrogate stock solutions were purchased in a concentration of 100 µg/ml. Water and acetonitrile (9:1, v/v) showed the best chromatographic response. Therefore, a five-point calibration curve was developed by diluting the stock solution to concentrations of 0.5, 1, 5, 10, 50 ng/mL in water and acetonitrile (9:1, v/v).

3.2.3 Chromatographic Conditions

The method development for quantification of the 7 pesticides and 2 surrogate standards employed an LC-MS/MS system triple quadrupole (Model 8040) mass spectrometer (Shimadzu Corporation, Kyoto, Kyoto, Japan) (Figure 2**Error! Reference source not found.**).

Figure 2 Triple Quadrupole (Model 8040)

A C18 stationary phase column (Kinetex® 1.7 µm EVO C18 100 Å 100 x 2.1 mm) was chosen for chromatographic separation based on previous published works (Rodrigues et al., 2007; Robles-Molina et. al., 2014) reproducible results using a similar column.

Four different mobile phases were compared for chromatographic elution. The four mobile phases included water, acetonitrile, water with 0.1% formic acid, and acetonitrile with 0.1% formic acid based on previous published work (Robles-Molina et al., 2014). Adding acid to the mobile phase is known have a beneficial effect on sensitivity when neutral/basic pesticides are analyzed in positive ion mode (Hu et al., 1999). Additionally, different flow rates (0.5 to 0.8 mL/min), column temperatures (40 to 60 ^{o}C), and solvent gradients were also taken into consideration.

3.2.4 Mass Spectroscopy Conditions

To achieve a favorable mass spectroscopy conditions for the target pesticides and surrogate standards, a full scan, SIM scan, and PI scan were performed to establish an optimum mass-to-charge (m/z) value and collision energy (CE) value. Data from the scans were compiled to make a MRM method. Additionally, three different ion sources and source needle position were also evaluated along with source parameters (DL temperature, nebulizing gas flow, heat block temperature, dry gas flow, and dwell time).

3.2.4.1 Full Scan Analysis

Full scan analysis was conducted for each of the target pesticides and surrogate standards using a concentration of 0.5 µg/ml. The full scan was run from 150 to 1000 m/z for all the compounds, individually. Positive and negative were considered when running a full scan in order to confirm and establish if the target analyte would produce a protonated, $(M+H)^{+}$, or

deprotonated, $(M-H)^-$, species. From previous published works, it was noted that a negative ion scan mode is best suited for 2,4-D, MCPA, MCPP, dicamba, and MCPA-13C6 since they produce a relative abundance of deprotonated ions, $[M-H]^-$ (Budde, 2004). While a positive ion scan mode was best suited for the target pesticides malathion, imidacloprid, carbaryl and imidacloprid-D4 since they would produce protonated ions, $(M-H)^+$ (Hu et al., 1999; Dujaković et al., 2010). The results generated an appropriate precursor ion m/z value for further optimization using a SIM scan.

3.2.4.2 SIM Scan Analysis

In order to optimize the m/z value for the precursor ion, a SIM scan analysis was performed for each of the target analytes and surrogate standards. In the SIM scan, a series of m/z values were evaluated. The results presented a specific m/z value for the precursor ion for each of the target analytes. The specific m/z value chosen was the ion species that was produced in abundance which generated the best results.

However, two of the target analytes had a similar optimize precursor m/z value and a similar retention time. In order to distinguish them from one another, a further fragmentation of the analytes was performed which produces a unique product ion.

3.2.4.3 PI Scan Analysis

A PI scan was performed to determine an m/z and CE for each analyte's product ion. The first set of CE values evaluated ranged from -10 V to 50 V in increments of 5 V. From the different CE values, an initial abundant m/z value for the product ions was produced. The m/z value was further optimized by establishing a new CE. Optimization was done by scanning a series of m/z values, ±0.5 the initial product ion scan's m/z value, and a series of CE values

ranging from ±5 V from the initial CE value. The results established an optimized m/z and CE value for accurate results. The PI scan provided a unique m/z value to increase the accuracy of the method.

3.2.4.4 MRM Analysis

MRM analysis combines selected dwell time with optimized peak-shape profiles, optimized precursor ion's m/z values, and the most abundant set of product ions with optimized CE and m/z values for each analyte. This analysis was made to obtain a method with high sensitivity.

3.2.4.5 Ion Source and Source Needle Position Analysis

The LCMS-8040 has the option of three ion sources. The ion sources available are electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and dual ESI/APCI. For optimization, a series of runs were evaluated, using the optimum parameters (section 2.2.3.1. thought 2.2.3.4), while the ion source was in ESI, APCI, or dual ESI/APCI.

Simultaneously, the source needle position was also established. Since the analytes ionize at different rates before entering the desolvation line, the position of the source needle needed to be considered to obtain the highest possible sensitivity of all the target analytes. The source needle was evaluated by adjusting it manually from 0mm to 5mm in increments of 1 mm.

3.3 Sample Pre-Treatment Method Development

3.3.1 SPE Pre-Treatment Method

SPE cartridges identified as potential pre-treatment method were Envi-Carb and Envi-18. Four different elution solvents were studied (Table 5). The cartridges were pre-conditioned with 10 mL of elution solvent then washed with 10 mL of ultra-distilled water. A 10mL sample,

spiked at 100 ng/mL, were then loaded onto the cartridges via a vacuum manifold at approximately 5 ml/min. Afterwards, the cartridges were washed with 10 mL of ultra-distilled water and excess water was removed by letting air pass through the cartridge for 10 min. The target analytes absorbed on the cartridge were eluted with 10 mL the appropriate elution solvent at a flow of approximately 1 mL/min. The eluted sample was reduced to dryness under nitrogen evaporation (section 3.3.4) and were reconstituted in a mixture of ultra-distilled water:ACN (9:1, v/v) to a volume of 10 mL. Additionally, the effluent was also tested to confirm that the cartridge's sorbent was effective.

Table 5 Elution Solvents

Envi-Carb
Methanol+acetonitrile(1:1)+1% ammonium hydroxide
Methanol+acetonitrile(7:3)+1% ammonium hydroxide
Envi-18
Methanol
Methanol+acetonitrile(7:3)+1% formic acid

3.3.2 LLE Pre-Treatment Method-A

A 250 mL spiked water samples, with a 10 ng/mL concentration, was transferred to a 500 mL separatory funnel and acidified with 3 mL of sulfuric acid (12 N). The water sample was extracted twice with 50 mL of diethyl ether. The extracts were then evaporated under nitrogen evaporation to dryness. The eluted sample was reduced to dryness under nitrogen evaporation (section 3.3.4) and were reconstituted in a mixture of ultra-distilled water:ACN (9:1, v/v) to a volume of 1 mL.

3.3.3 LLE Pre-Treatment Method-B

Method-B used a different solvent. A 250 mL spiked sample was acidified to a pH of 5.5-6 using hydrochloric acid (HCl). The samples were transferred to a 500 mL separatory funnel.

The water sample was extracted twice with 50 mL of dichloromethane (DCM). The extracts were then evaporated under nitrogen evaporation to dryness. The eluted sample was reduced to dryness under nitrogen evaporation (section 3.3.4) and were reconstituted in a mixture of ultra-distilled water:ACN (9:1, v/v) to a volume of 1 mL.

3.3.4 Evaporation

Experiments were conducted to confirm that there was no loss in recovery during nitrogen evaporation. Spiked 20 mL samples in an organic solvent (DCM or diethyl ether) were transferred to a 32 mL vial and placed in the nitrogen evaporation system. The organic layer was evaporated to dryness. Once the vials were fully evaporated, 1 mL of ultra-distilled water:ACN (9:1, v/v) was added and transferred to an analytical.

3.4 Method Validation

3.4.1 Experiment Design

This study utilized two surrogate standards to verify the recovery from environmental samples. MCPA-13C6 and Imidacloprid-D4 were selected as surrogate standards for the target analytes because of their structural similarities to two of the target pesticides. Five levels of calibration solution were prepared by diluting the stock solution. Experiments were performed in two experimental runs for each target analyte calibration solution using analyte concentration ranging from 0.5 to 50 ng/mL to examine method linearity. A series of five-point calibration curves were established. Method linearity was quantified by the R^2 value of the linear regression curve. The calibration curves showed acceptable linearity.

For the method developed, limit of quantitation (LOQ) for each target analyte was determined. The LOQ is defined as the lowest analyte concentration that can be precisely

measured by the method (Armbruster et al., 2008). LOQ was estimated using standard-deviation/slope ratio based on the signal-to-noise ratio using equation 1, where δ is the standard deviation of the calibration curve intercepts, and S is the mean slope of the calibration curves (Ravisankar et al., 2015, Araujo, 2005).

$$LOQ = 10 \times \ \delta/S \qquad (1)$$

Once the quantitative method was validated, the reliability of the liquid-liquid extraction procedure was evaluated by conducting a series of recovery experiments. Specifically, LC-MS/MS experiments were performed with known concentrations of target analytes to ensure that the samples are recovered when analyzed. The recovery experiments were performed by spiking known concentrations of target analytes at 10 ng/mL. The experiment process is shown in Figure 3 and further detailed in section 4.3.

Results of the recovery experiment were compared with the known concentrations of the spiked samples to determine the accuracy as percent recovery (%R), and precision as standard deviation of %R, of the procedure. %R was calculated using equation 2.

$$\%R = \frac{Spiked\ samples\ concentration}{Spiked\ standard\ concentration} \qquad (2)$$

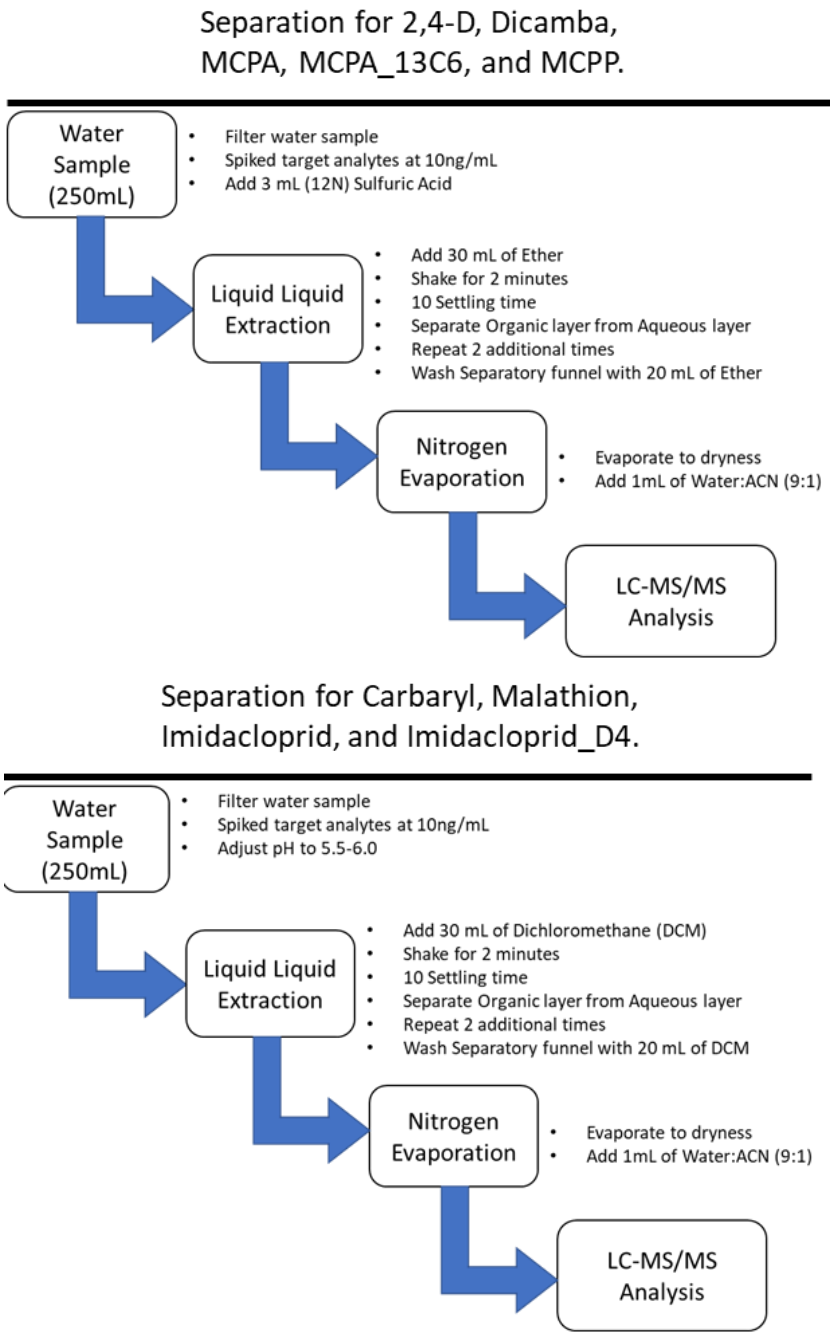


Figure 3 Experimental process for method validation

3.5 Results and Discussion

3.5.1 Optimization of Chromatographic Conditions

Acetonitrile and water (9:1, v/v) was selected as the best solvent matrix to use for the target and surrogate analyte standards since it showed the most effective chromatography response. A Kinetex® EVO C18 (100 Å, 100 x 2.1 mm, 1.7 µm) was used for this study which produced excellent peak retention time and resolution.

Among the four combinations of mobile phase solvents, the optimum chromatography was achieved with mobile phases of acetonitrile with 0.1% formic acid and water with 0.1% formic acid. Flow rate, column temperature, gradient conditions that displayed the optimum peak shape, response, and resolution are shown in Table 6. For the elution from the stationary phase, the initial composition, acetonitrile with 0.1% formic acid, was 10% which increased linearly to 80% in 4 minutes. At 4.1 minutes, the concentration was reduced to 10% for a duration of 2.9 minutes in order to re-equilibrate the column to initial conditions. In total the analysis per sample was 7 minutes. The analytes were detected from 1.75 to 3.6 minutes and the column was allowed to re-equilibrate to initial conditions from 4.1 to 7 minutes.

Table 6 Optimized Chromatographic Conditions

Column	Kinetex® 1.7 µm EVO C18 100 Å 100 x 2.1 mm	
Mobile phase A (MP_A)	Water (0.1% Formic Acid)	
Mobile phase B (MP_B)	Acetonitrile (0.1% Formic acid)	
Pump parameters	Time (min)	MP_B%
	0	10
	4	80
	4.1	10
Flow rate	0.6 mL/min	
Run time	7 minutes	
Column temperature	45°C	
Injection volume	50 µL	
Injection wash solvent	Water:Acetonitrile (1:1)	

3.5.2 Optimization of Mass Spectrometry Conditions

While using the parameters in Table 7, the best sensitivity was achieved for all the target analytes simultaneously.

Table 7 MS Conditions

MS Conditions	
Ion Source	Dual (ESI and APCI)
Nebulizing Gas Flow (L/min)	2
DL Temperature	250 ^{o}C
Heat Block Temperature	400 ^{o}C
Drying Gas Flow (L/min)	15
Dwell time (msec)	60
Source Needle Position	3mm
*other parameters as per tuning file	

3.5.3 Results of Qualitative Scan Analysis

As part of the MRM method development, each of the target analytes underwent a series of scans (full, SIM and PI) to optimize mass spectrometry conditions for suitable scan mode, m/z, and CE values for the precursor and product ions. The full mass spectrum for each analytes SIM, PI, and optimized PI scans are presented in Appendix A.

A full scan analysis, in negative scan mode, produced deprotonated ion, $(M-H)^-$, species for 2,4-D, MCPA, MCPP, dicamba, and MCPA-13C6. While a positive scan mode was best suited for the target pesticides-malathion, imidacloprid, carbaryl and imidacloprid-D4 since they produced protonated ions, $(M-H)^+$, species. The protonated or deprotonated ion were chosen as the initial precursor ion for their respected target analyte.

In order to optimize the precursor ion, a series of SIM scans where conducted. The SIM scan mass spectrum analysis is presented in Figure A1 and Table A1. The SIM scan provided an optimum m/z values shown in **Error! Reference source not found.**.

PI scans were performed to target an appropriate m/z values and a CE for the product ions of the target analytes. The PI scan mass spectrum analysis is presented in Figure A2 to Figure A5 and Table A2**Error! Reference source not found.** to Table *A5*. The multiple m/z values, from the PI scan, were narrowed down to one single m/z value for each of the target analyte, which can be found in Table 8.

In order to further increase sensitivity, the ion source and source needle position was also considered. The results of establishing the appropriate ion source and needle positions are presented in Table 9. When comparing ESI and APCI, ESI showed a greater sensitivity in detecting the target analytes for both positive and negative ions. When comparing ESI and dual (ESI and APCI), the negative ions have a similar sensitivity. However, dual ion source displayed a significant increase in sensitivity for positive ions. Therefore, in order to simultaneously optimize the sensitivity for all target analytes, the data collected from the series of scans using ESI, APCI, and dual (ESI and APCI), while at the same time moving the source needle's position, concluded that dual ion source (ESI and APCI) was the best option. The best two needle positions were at 2 and 3 mm. At needle position 2mm, the negative ions showed greater sensitivity compared to position 3 mm, but the positive ions sensitivity was remarkably low with respect to position 3 mm. At position 3 mm, positive ions displayed a significant increase in sensitivity while negative ions displayed a slight decrease in sensitivity. Overall, dual ESI/APCI and 3 mm are the favorable parameters suited for the ion source and needle position, respectively.

The results of the full, SIM, and PI scans, ion source, and source needle position experiments were combined to develop an MRM analysis. Optimized target analytes were

setup in the data acquisition system to conduct quantitative experiments. Details of further MRM condition optimization are presented in Table 8.

The product ion chromatograms of each target analyte, presented in Figure 4, show well defined peak shapes and target analyte separation. As previously mentioned, two of the target analytes, and one surrogate standard, appeared to have identical retention time. However, since they each produce a unique product ion and therefore distinguishable. Additionally, blank samples at the beginning of the run were shown to have signal peaks but did not appear to interfere with any of the retention times of any of the target analytes.

Table 8 MRM data acquisition parameters

		Analyte	Retention Time (min)	Precursor Ion	Product Ion	Collision Energy (V)
Target Analyte	1	Carbaryl	2.75	201.5	145.2	-11.0
	2	Imidacloprid	1.75	255.5	209.0	-14.0
	3	Malathion	3.60	330.5	127.05	-13.0
	4	MCPA	2.80	199.2	141.1	12.0
	5	MCPP	3.10	213.2	141.1	13.0
	6	2,4-D	2.80	219.1	161.0	12.0
	7	Dicamba	2.40	219.1	175.1	8.0
Surrogate Standard	8	MCPA-13C6	2.80	205.0	147.1	13.0
	9	Imidacloprid-D4	1.75	259.9	213.0	-15.0

Table 9 Ion source and needle position results

Needle position (mm)	Compound (m/z)						
	Carbaryl (145.2)	Imidacloprid (209.0)	Malathion (127.05)	MCPP (141.1)	MCPA (141.1)	Dicamba (175.1)	2,4-D (161.0)
	Absolute Intensity						
ESI (Ion Source)							
0	1235	2470	2470	60319	21805	14116	40511
1	12	336	767	69902	27221	17307	53850
2	275	1105	3342	80423	28961	19256	54757
3	2490	5565	18428	60404	21061	12712	37239
4	1470	5908	8339	19314	7069	4088	13075
5	1243	4944	5210	14068	5642	2966	10509
APCI (Ion Source)							
0	436	576	3489	20455	6039	3201	7874
1	261	172	1896	40195	9129	6481	12963
2	397	238	2618	39876	12705	8327	15275
3	578	932	2679	16796	5450	3170	7899
4	229	991	984	7392	1957	945	2908
5	0	0	0	0	0	0	0
Dual (ESI/APCI) (Ion Source)							
0	1617	3475	14833	69176	22920	15004	38724
1	199	495	1378	81925	29980	19329	58595
2	255	1783	3045	88380	33327	20968	66976
3	7973	8903	24893	76080	25387	14355	46817
4	3293	11542	13999	28022	9690	5536	17956
5	1663	8939	7774	14489	5311	3229	10586

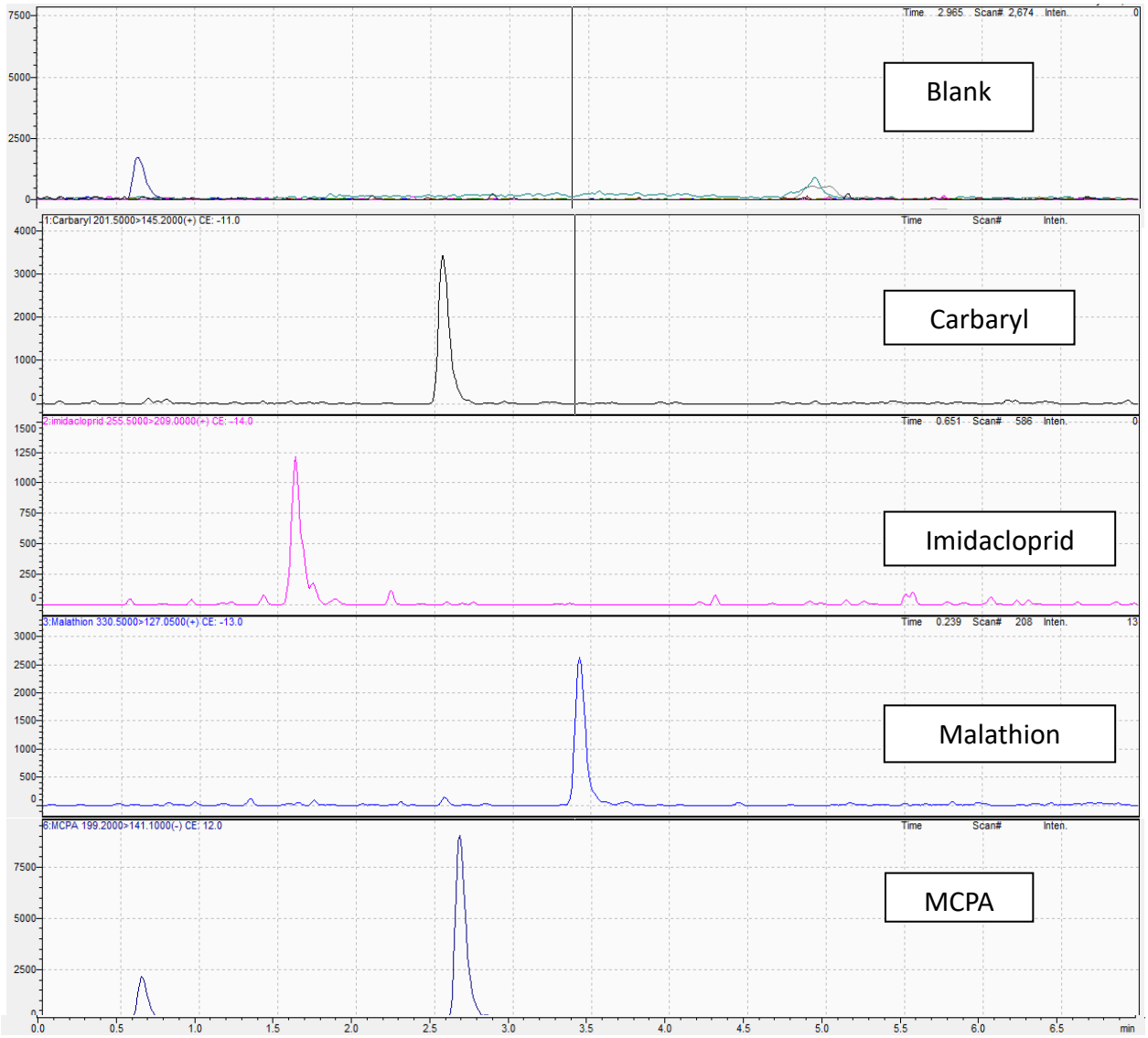


Figure 4 Chromatograms of target analytes at concentration of 5 ng/mL (Signal Intensity vs Retention time)

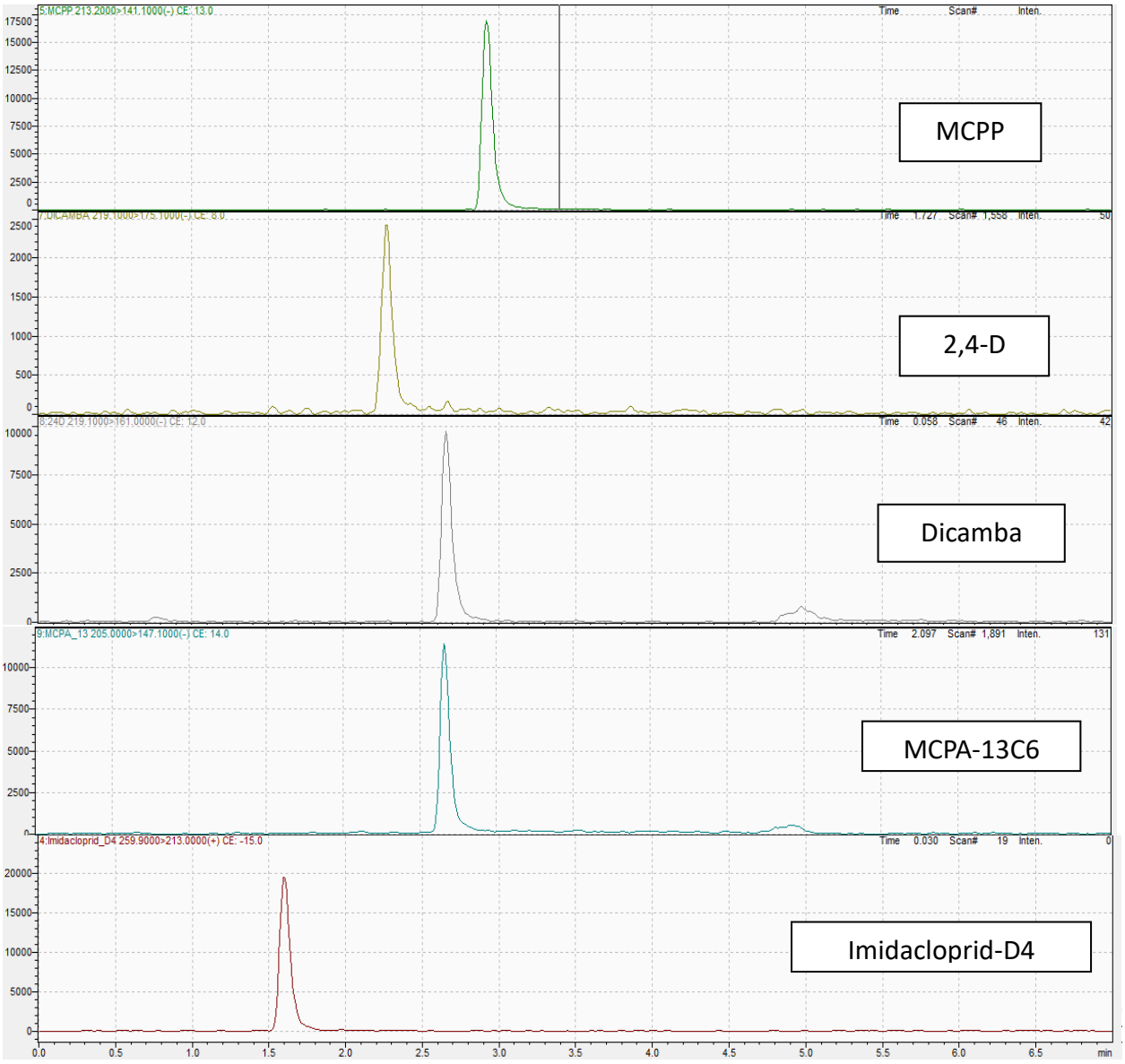


Figure 4 (cont.) Chromatograms of target analytes at concentration of 5 ng/mL ((Signal Intensity vs Retention time)

3.5.4 Pre-treatment Results

3.5.4.1 SPE Pre-Treatment Result

Two different SPE cartridges were examined for the effectiveness of the sorbent to capture the target analytes. The effluent results were generally desirable (Table 10). Most of the results were mostly below 11% with only two compounds above 50%. A low concentration in the effluent indicates that the analytes were successfully adsorbed by the cartridge sorbent. However, the elution recovery was not as desirable (Table 11). The elution recovery indicates if the elution solvent was successful in flushing the analytes from the sorbent. The elution recoveries were generally below 15% for all compounds with the only two analytes above 50%.

Table 10 SPE Effluent Results

Pesticide	% Effluent			
	Envi-Carb		Envi-18	
	MeOH:ACN (1:1:1%AH)	MeOH:ACN (7:3:1%AH)	MeOH	MeOH:ACN (7:3:1%FA)
Carbaryl	50.0	11.3	0.0	0.0
Imidacloprid	11.0	7.7	1.1	0.0
Malathion	0.0	0.0	0.0	0.0
MCPP	0.0	0.0	5.5	0.0
MCPA	0.0	0.0	6.8	0.0
Dicamba	0.0	0.0	67.9	8.2
2,4-D	0.0	0.0	0.0	3.9

Table 11 SPE Recovery Results

Pesticide	% Recovery			
	Envi-Carb		Envi-18	
	MeOH:ACN (1:1:1%AH)	MeOH:ACN (7:3:1%AH)	MeOH	MeOH:ACN (7:3:1%FA)
Carbaryl	14.8	10.0	2.8	5.9
Imidacloprid	0.0	7.1	1.8	3.6
Malathion	0.0	0.0	0.0	8.2
MCPP	0.0	0.0	5.5	4.4
MCPA	0.0	0.0	6.8	4.7
Dicamba	53.8	56.0	2.8	3.6
2,4-D	0.0	0.0	7.0	3.9

3.5.4.2 LLE Pre-Treatment Results

Results of the recovery study for Method-A, using diethyl ether, and Method-B, using DCM, are presented in Table 12. From the results, Method-A was best suited for recovery of the analytes that produce a negative ion, 2,4-D, dicamba, MCPA, and MCPP, during LC-MS/MS analysis. Using method-A, the negative ion producing pesticides displayed a recovery above 75%. However, the positive ion-producing pesticides displayed recovery under 40%.

Meanwhile, method-B was best suited for recovery of the analytes that produce a positive ion, carbaryl, imidacloprid, malathion, during LC-MS/MS analysis. Using Method-B, the positive ion producing pesticides displayed a recovery above 75%. Furthermore, using Method-B, the negative ion-producing pesticides displayed recovery under 35%.

Due in part to time constrains, our attempts of reproducing an effective SPE method were unsuccessful, and therefore two separate LLE methods were implemented as a pre-treatment method since they were consistently reproducible for the target analytes. Section 4.3 details the preconcentration methods established for the study.

Table 12 LLE Results

Pesticide	% Mean Recovery (std)	
	Method-A	Method-B
Carbaryl	39.7 (29.9)	80.0 (3.9)
Imidacloprid	0 (0)	99.2 (12.3)
Malathion	26.8 (19.6)	79.1 (1.3)
MCPP	93.7 (10.7)	31.0 (13.7)
MCPA	102.0 (13.6)	32.4 (10.3)
Dicamba	78.7 (9.9)	1.5 (0.4)
2,4-D	91.9 (13.6)	15.0 (4.4)

3.5.4.3 Evaporation Results

Evaporation experiments were conducted to confirm that there was no loss in recovery during nitrogen evaporation. Results indicate that there was not a significant loss during the evaporation step of the SPE or the LLE methods (*Table 13*).

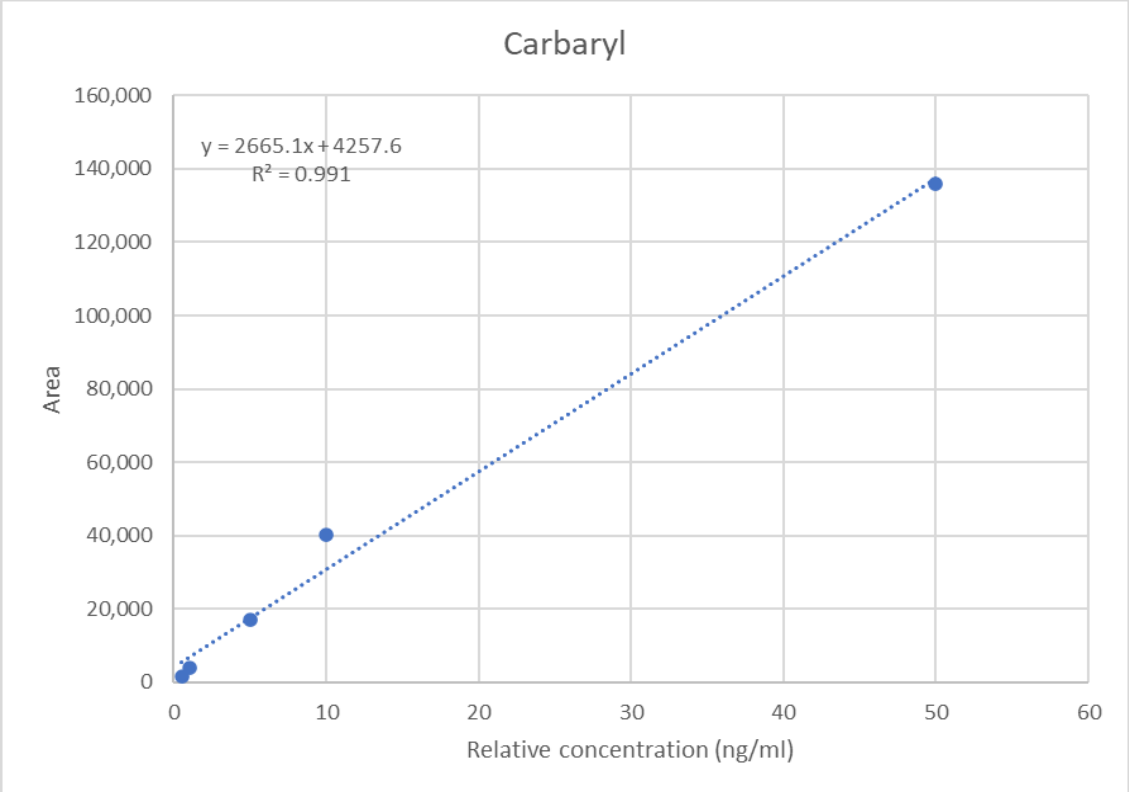
Table 13 Evaporation Results

Pesticide	Recovery (%)
Carbaryl	95.5
Imidacloprid	99.2
Malathion	95.4
MCPP	87.2
MCPA	95.0
Dicamba	96.3
2,4-D	102.9

3.5.5 Method Validation Results

As shown in Figure 5, the obtained calibration curves indicate acceptable linearity, with R^2 values greater than 0.99. LOQ is the lowest analyte concentration that can be precisely measured. The LOQ was calculated for both the instrument and the method. The instrument LOQ was established based on the concentrated samples and the lowest concentration of analyte that could be precisely measured by the instruments. The method LOQ value is the lowest concentration of analyte that could precisely be measured based on a 250mL sample. LOQ values were determined for each target analyte and are presented in Table 14.

Results of the recovery study are presented in Table 15. The mean recovery obtained from the samples spiked at 10 ng/mL was 83.2% ±12.56%, The recovery ranged from 61.08% to 107.33%.



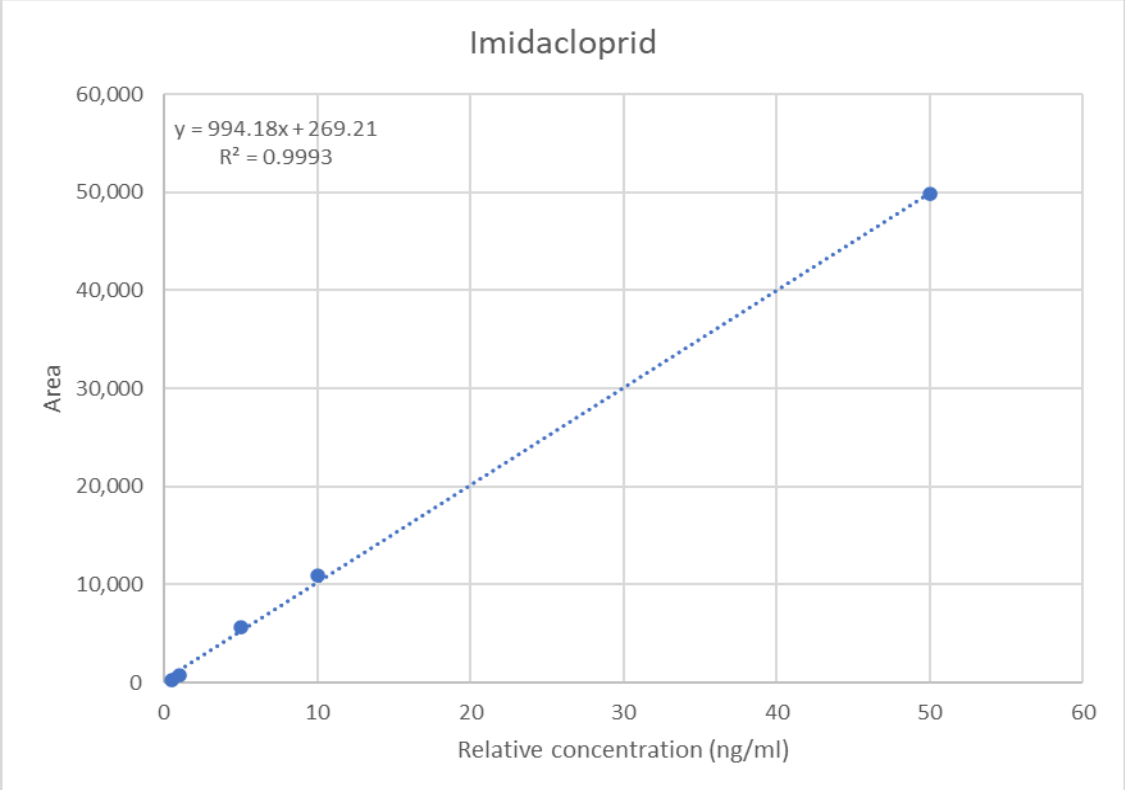
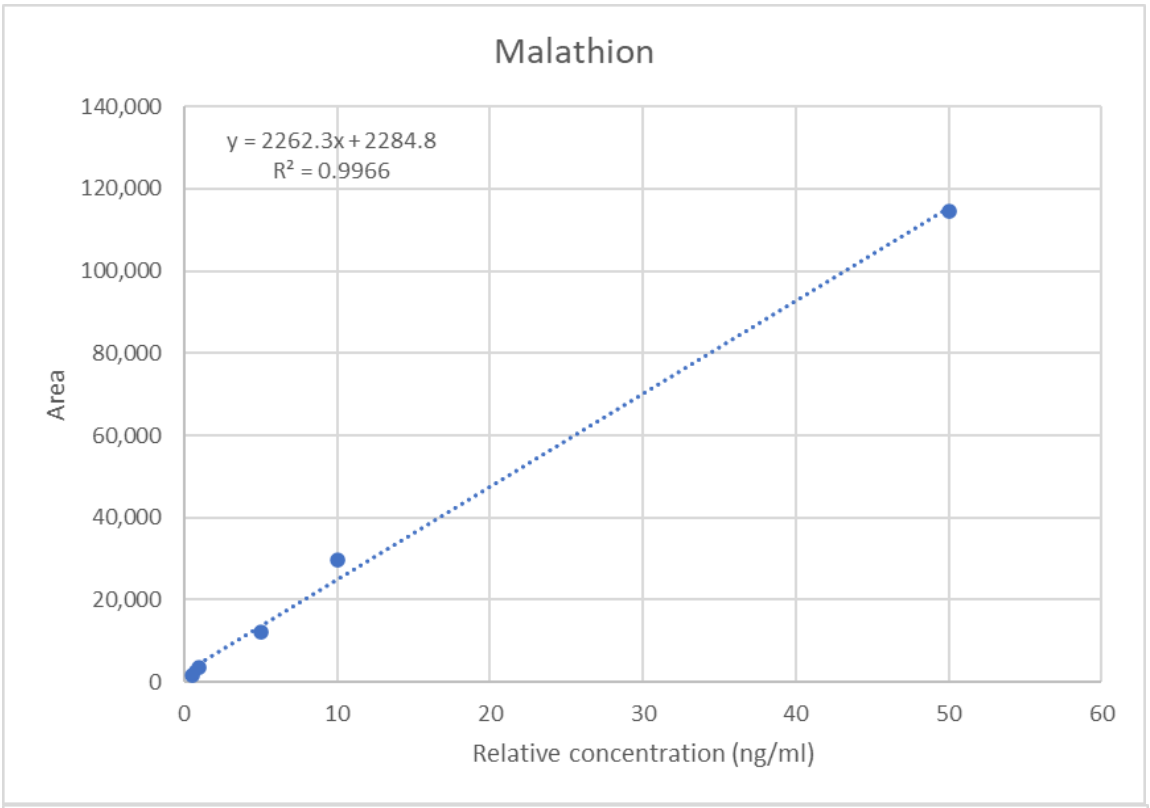


Figure 5 Calibrations Curve for Target Analytes



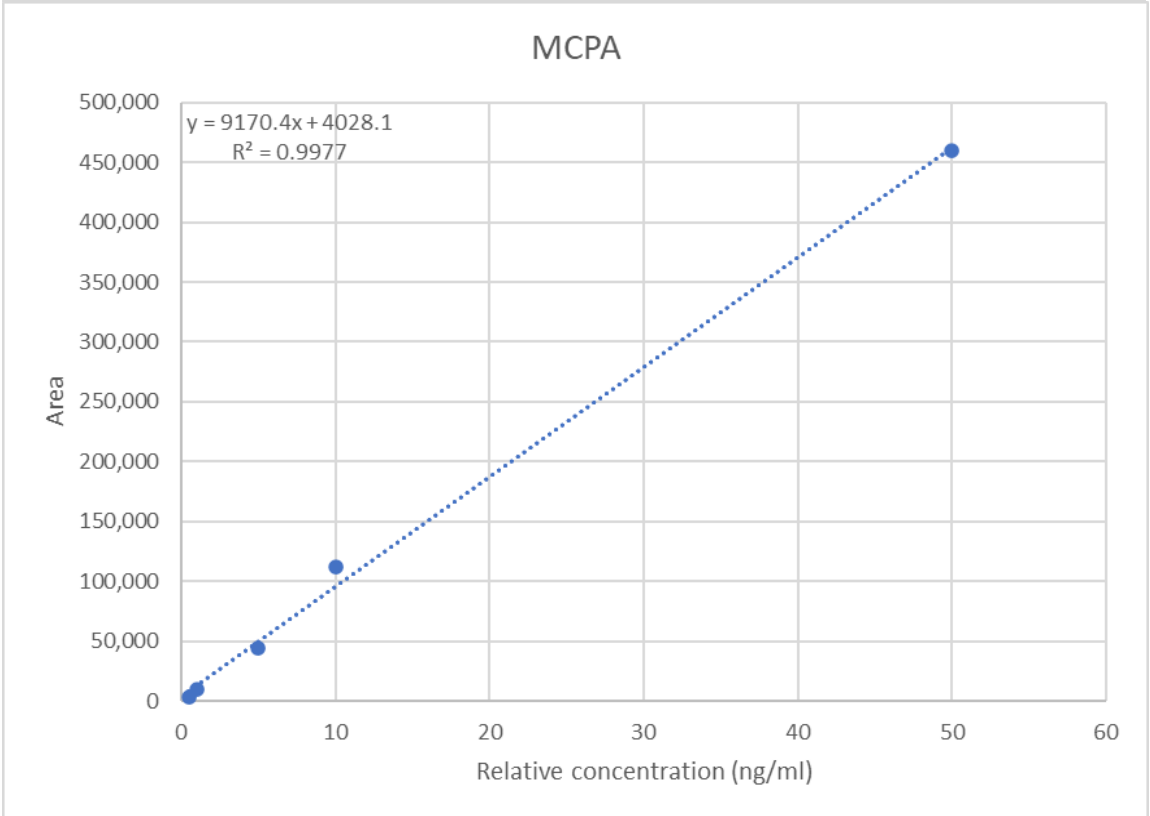


Figure 5 (cont.) Calibrations Curve for Target Analytes

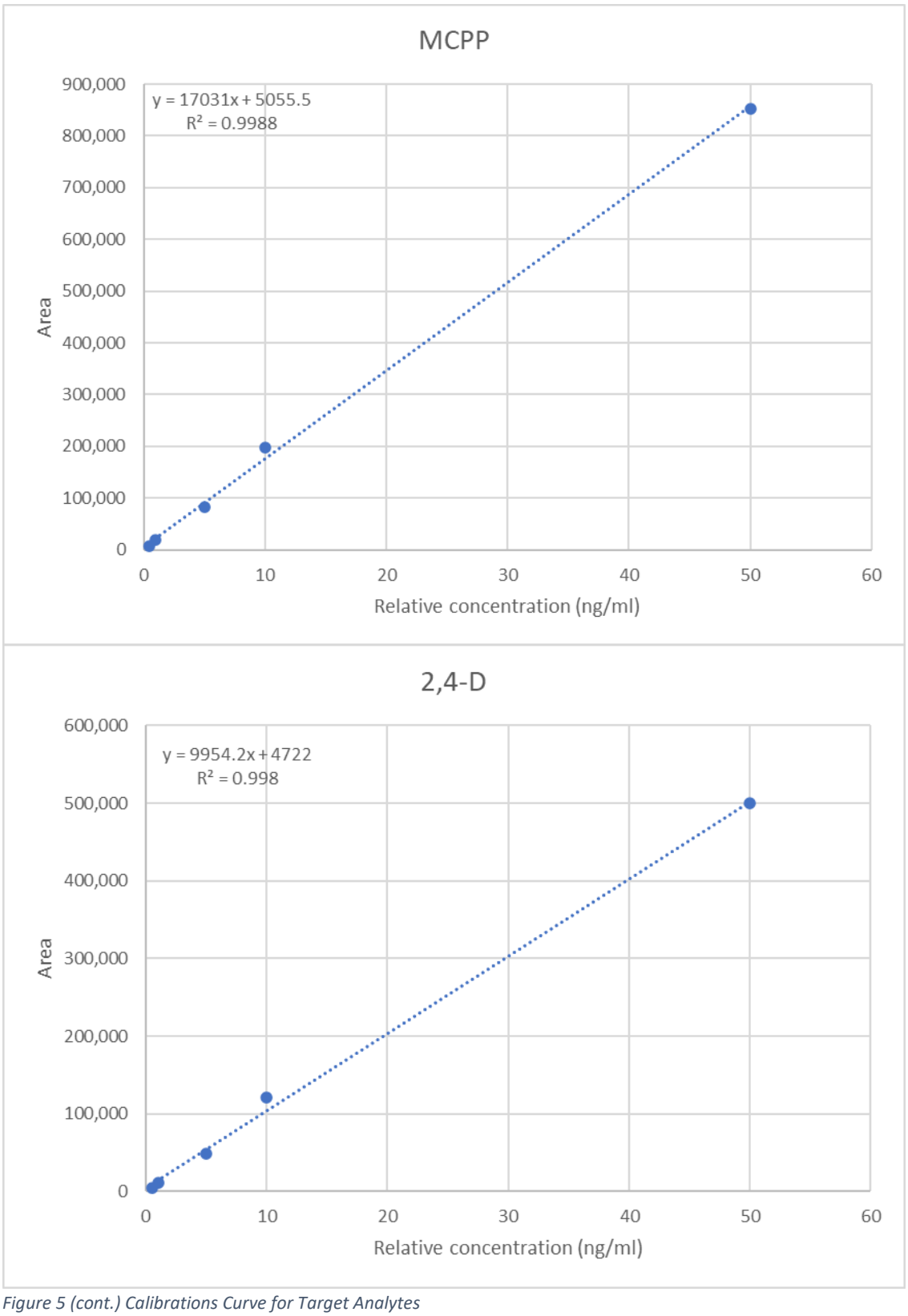
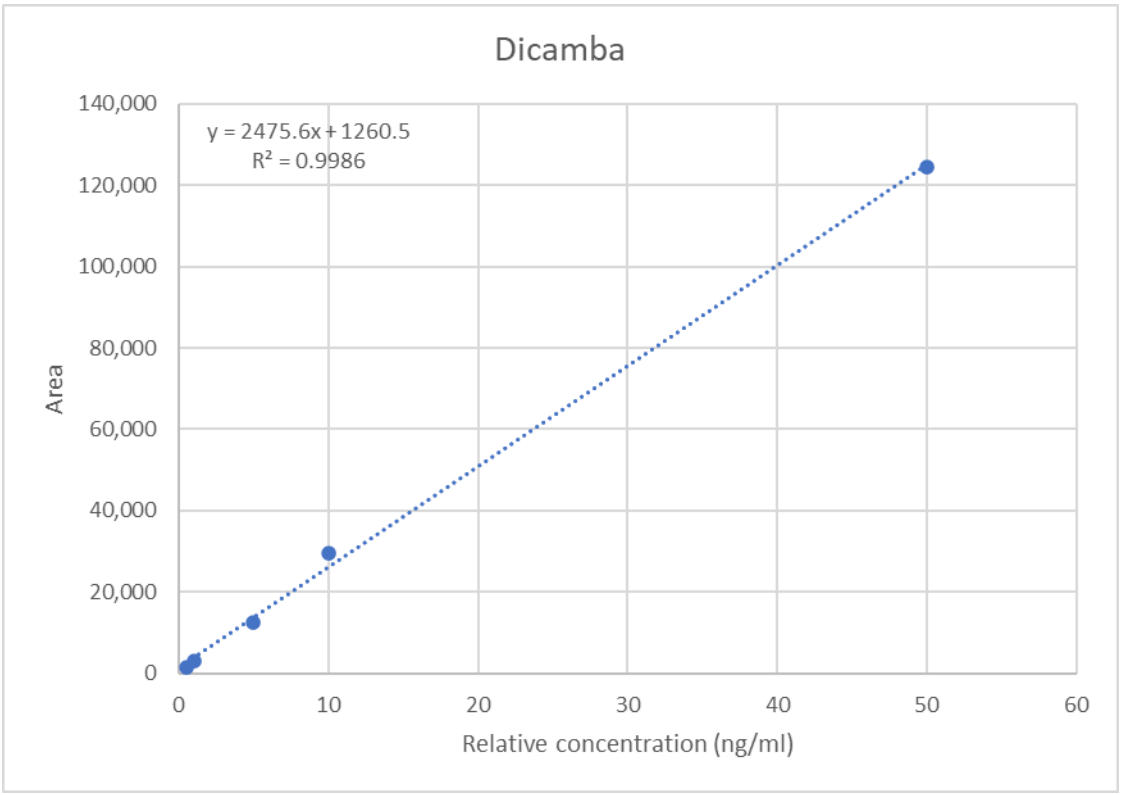


Figure 5 (cont.) Calibrations Curve for Target Analytes



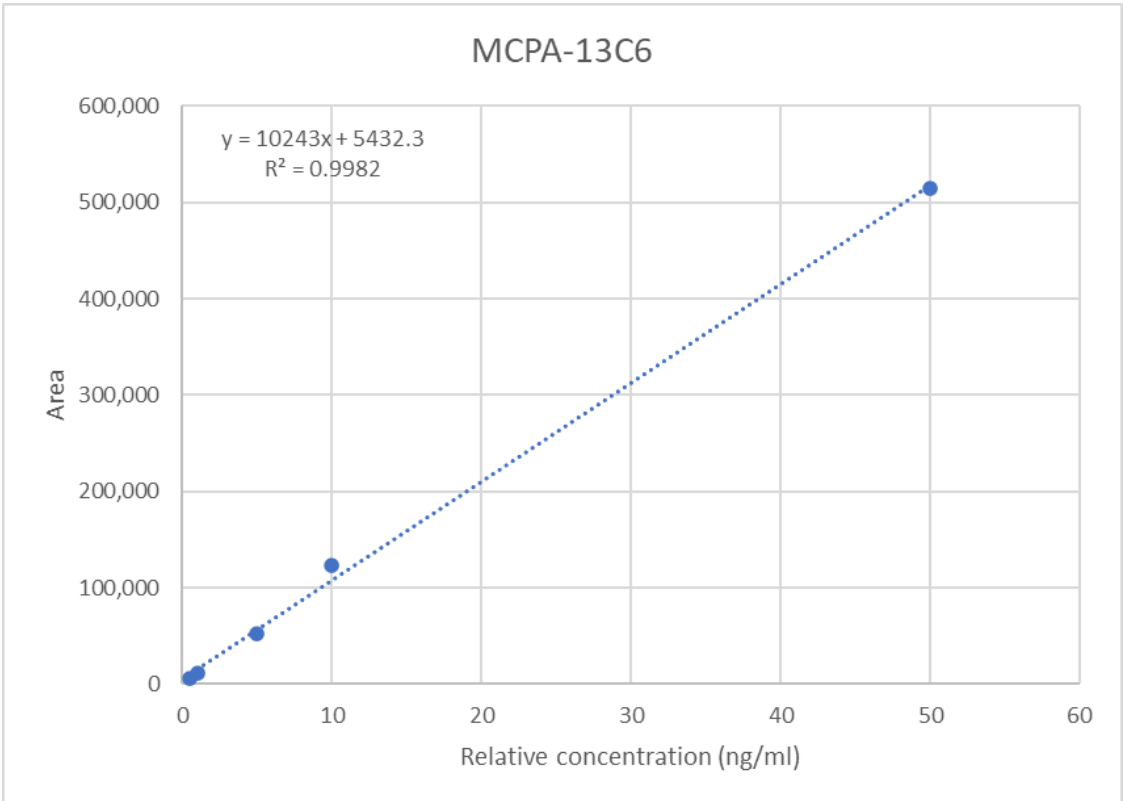


Figure 5 (cont.) Calibrations Curve for Target Analytes

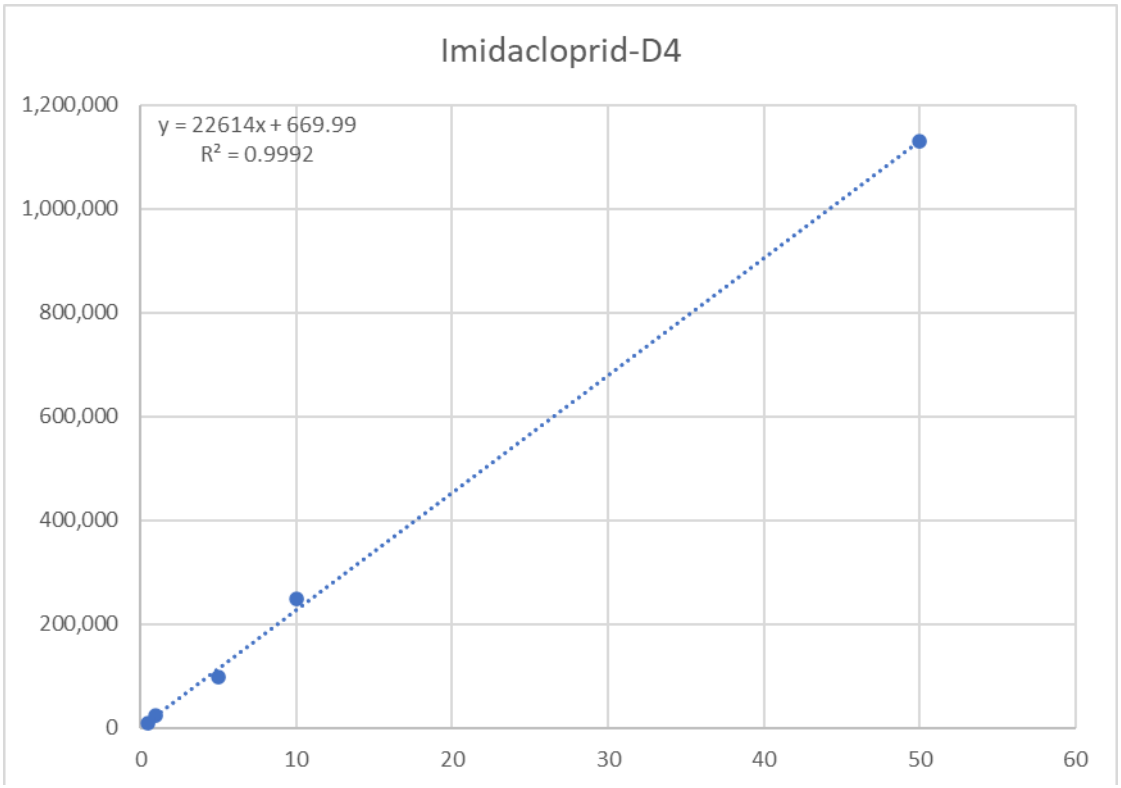


Figure 5 (cont.) Calibrations Curve for Target Analytes

Table 14 LOQ for Target Analytes

Target Analytes		Instrument LOQ (ng/mL)	Method LOQ (ng/mL)
1	Carbaryl	8.1	0.032
2	Imidacloprid	7.4	0.030
3	Malathion	2.0	0.008
4	MCPA	0.3	0.001
5	MCPP	0.5	0.002
6	2,4-D	1.1	0.004
7	Dicamba	1.6	0.006
8	MCPA-13C6	1.0	0.003
9	Imidacloprid-D4	1.0	0.003

Table 15 Percent Recovery

	1		2	
Analyte	Recovery (%)	Std(%)	Recovery (%)	Std(%)
Carbaryl	107.3	6.0	61.1	3.6
Imidacloprid	73.2	0.9	99.2	12.3
Malathion	91.2	4.3	79.2	1.3
MCPA	69.9	0.1	97.9	1.9
MCPP	75.3	1.5	91.5	2.4
2,4-D	101.8	2.0	85.7	2.1
Dicamba	71.3	3.9	75.9	2.8
MCPA-13C6	73.2	2.1	85.2	1.3
Imidacloprid-D4	71.5	2.3	87.7	1.7

CHAPTER 4: APPLICATION OF A QUANTITATIVE METHOD ON ENVIRONMENTAL SAMPLES USING LC-MS/MS

4.1 Introduction

It is estimated that around 25 percent of Wisconsin residents obtain their drinking water from over 800,000 private wells (DNR, 2017). In a 2016 sampling effort across Wisconsin, the DATCP sampled 401 private drinking wells and tested them for pesticides and their metabolites. The results showed that 41.7% of the selected wells were shown to have a detectable concentration of pesticides and their metabolites, which showed a rise from 33.5% from a 2007 survey (DATCP, 2017). However, the survey conducted by the DATCP used a stratified random sampling approach. Entirely covered urban, non-agricultural land and water land were excluded from sampling (DATCP, 2017).

The Milwaukee metropolitan area was used as the study area. Milwaukee metropolitan area is the largest metropolitan area in Wisconsin and ranks the 39th largest metropolitan area in the United States. Groundwater samples were collected from private wells that provide drinking water for residential or commercial use. The wells are located primarily in Milwaukee (i.e. Wauwatosa and Franklin), Ozaukee (i.e. Mequon and Grafton), Washington (i.e. Hubertus, Germantown and Richfield), and Waukesha (i.e. Muskego and Elm Grove). There were 16 locations in total that were willing to participate in this study. Each of these locations are represented below in Figure 6. Each of the samples were chosen based primarily as a function of the location within a well-kept neighborhood in the sand and gravel or dolomite aquifer. Furthermore, the wells chosen were relatively shallow at approximately 100 ft, with the exception for wells 2, 4, and 8. 100 ft well depth was chosen under the hypothesis that shallow

wells would be more susceptible to pesticide contamination. Furthermore, all the locations, apart from Well 7 and 15, recognized applying some form of pesticides (fungicides, insecticides, herbicides) to their lawn multiple times a season either personally or through a company.

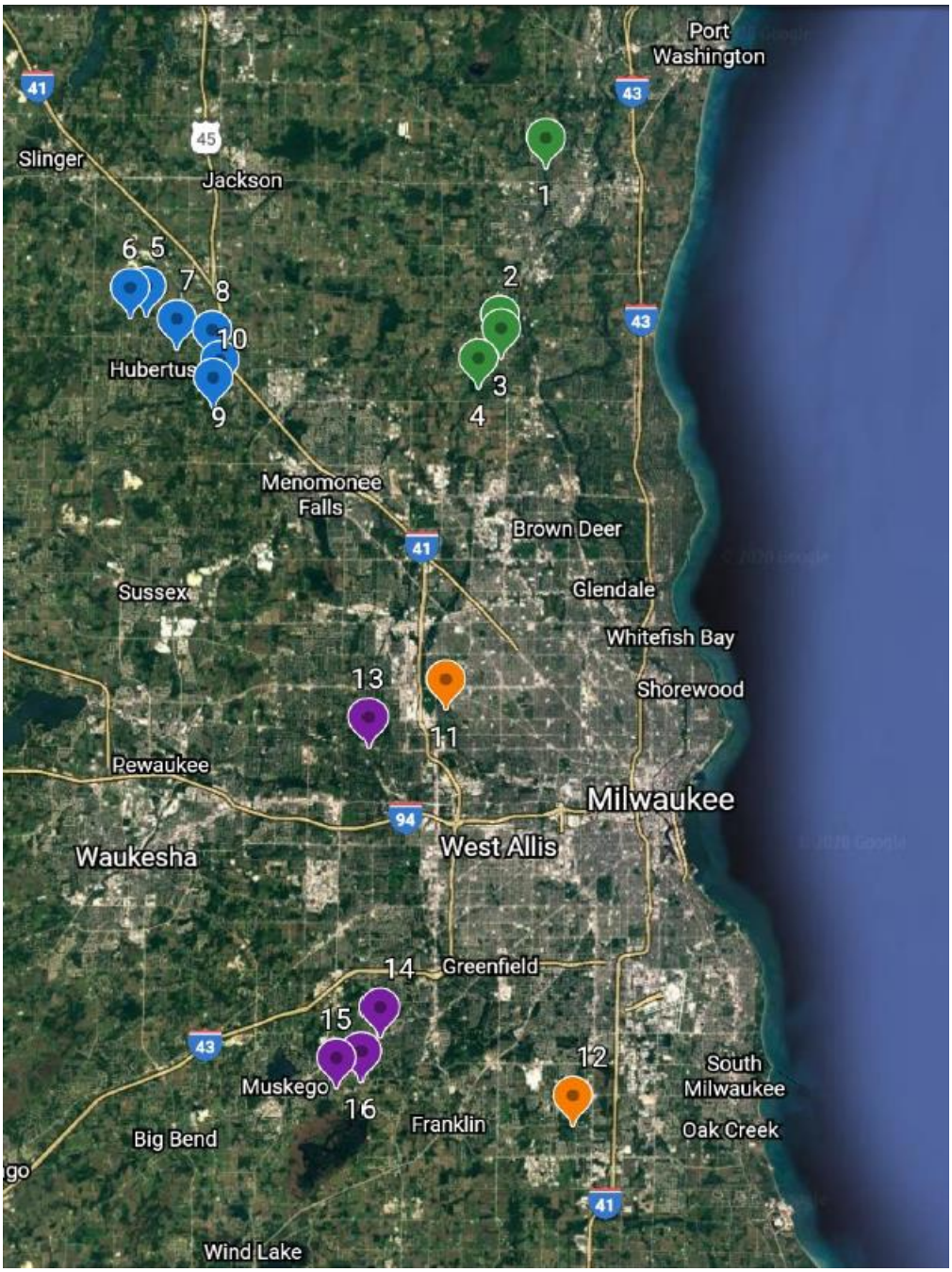


Figure 6 Google Earth Screenshot of Well Locations

Blue: Washington County Green: Ozaukee County

Purple: Waukesha County Orange: Milwaukee County

4.2 Sample Collection

The optimized and validated LC-MS/MS method was applied to groundwater samples collected from various locations in southeastern Wisconsin on different sampling events (June-July, August, November 2019, and February 2020).

During each sampling event, samples were collected in amber glass containers from each location. Water samples were collected in duplicate (2, 1 L samples) from the spigot just before the water pump within each of the homeowner's basement. Immediately following collection, all samples were stored in coolers on ice (approximately 4 °C), transported to the laboratory, and stored at 0 °C until analyzed. As extra precaution, each bottle was wrapped in tin foil.

4.3 Sample Preparation

Prior to sample cleanup and extraction, water samples were removed from storage and allowed to naturally reach room temperature. From the 1-liter samples collected, two separate 250ml sample were measured. The samples were prepared for analytical analysis by LLE with two organic solvent: DCM and diethyl ether. The samples were passed through a Whatman® GF/B glass microfiber filter (1µm) to remove suspended solids using micro-filtration under vacuum. One 250 mL sample was set aside for LLE using diethyl ether. The second 250ml was set aside for LLE using dichloromethane. DCM was used for the positive ion analysis of carbaryl, imidacloprid, malathion and surrogate standard imidacloprid-D4. Diethyl ether was used for the negative ion analysis of 2,4-D, dicamba, MCPA, MCPP and MCPA-13C6. After filtration, 0.5 mL at a concentration of 100ppb of the surrogate standards were added to both 250mL samples.

For the negative ion analysis analytes, the filtered water samples were transferred to a 500 mL separatory funnel and acidified with 3 mL of sulfuric acid (12 N). After the water sample was transferred, 20 mL of diethyl ether was used to wash the filtering flask and then transferred into the separatory funnel. Once the sample, and 20 mL of wash, were in the separatory funnel an additionally 30 mL of diethyl ether (total of 50 mL) was added. The funnel was then hand-shaken for a minimum of 2 minutes and allowed to settle for 10 minutes. Afterwards, the aqueous and organic layers were separated into different flasks. The organic layer was transferred to a 600 mL nitrogen evaporation flask. The aqueous phase was transferred back into the separatory funnel and the process was repeated 2 additional time by only adding 30 mL of diethyl ether, without adding 3ml of sulfuric acid. After the third organic layer transfer, the aqueous layer was drained into a hazardous waste container. The, now emptied, funnel was washed using 20 mL of diethyl ether and added to the 600 mL nitrogen evaporation flask. A total of 130 mL of diethyl ether was used.

For the positive ion analysis analytes, the 250mL filtered samples where acidified to a pH of 5.5-6 using hydrochloric acid (HCl). The filtered water samples were transferred to a 500 mL separatory funnel. Afterwards 20ml of dichloromethane was used to wash the filtering flask into the separatory funnel. Once the sample, and 20ml of wash, were in the separatory funnel an additionally 30 mL of DCM (total of 50 mL) was added. The funnel was then hand-shaken for a minimum of 2 minutes and allowed to settle for 10 minutes. Afterwards, the aqueous and organic layers were transferred into different flasks. The organic layer was transferred to a 600 mL nitrogen evaporation flask. The aqueous phase was transferred back into the separatory funnel and the process was repeated two additional time by only adding 30 mL of DCM. After

the third organic layer transfer, the aqueous layer was drained into a hazardous waste container. The, now emptied, funnel was washed using 20 mL of DCM and added to the 600 mL nitrogen evaporation flask. A total of 130 mL of DCM was used.

Afterwards, using Labconco RapidVap© N_2/48 Evaporation system, the organic layers were evaporated to dryness using the setting found in Table 16 for 600 mL flask. Afterwards, 20 mL of either diethyl ether or DCM, whichever was appropriate for the samples, was added to the 600 mL flask and transferred to a 32 mL vial. The 32 mL vial was then placed in the nitrogen evaporation system and the organic layer was evaporated to dryness using the setting found in Table 16 for 32 mL vial. Once the vials were fully evaporated, they were taken out of the evaporation system and allowed to cool for 1 minute. After that, 1 mL of water:acetonitrile (9:1, v/v) was added and afterwards transferred to an analytical vial and stored at 4 ^oC until analyzed by the developed LC-MS/MS method.

Table 16 Nitrogen Evaporation Settings

Flask/Vial	Temperature (^oC)	Nitrogen Pressure (psi)	Speed Vortex (%)	Sample Setting	Duration (min)
600 mL	45	15	70	8	20-25
32 mL	45	15	60	2	30-35

4.4 Application of LC-MS/MS Method

The developed LC-MS/MS method described earlier in this thesis was used to identify the 7 target pesticide analytes in groundwater samples collected from the southeastern Wisconsin wells. The concentration for each compound detected in a sample injected into the LC-MS/MS system was calculated using a linearly regressed, five-point calibration curves relating sample concentration to instrument response. The actual concentration for each detected target analyte was calculated using equation (3), where c_f is the final concentration in

the 1 L groundwater sample (ng/mL), c_i is the concentration in the injected sample (ng/mL), v_1 is the volume in the sample vial (mL), and v_2 is the volume of the groundwater sample (mL):

$$c_f = \frac{c_i \times v_1}{v_2} \qquad (3)$$

4.5 Results and Discussion

A total of sixteen groundwater wells (**Error! Reference source not found.**) were sampled in southeastern Wisconsin during 4 sampling events in June-July, August, and November 2019 and February 2020. However, not all of well locations were sampled during all four events due to scheduling conflict with willing participants, time constrains or other unforeseen circumstances. During the first round of sampling, in June-July 2019, groundwater was collected from well locations 1-6, 9, 11-16. The second round, sampled in August 2019, included groundwater samples from well locations 1-16. In November 2019, the third sampling event included groundwater samples from well locations 2,5,7-15. During the final sampling event, in February 2020, groundwater samples were collected from well locations 2-3, 5, 7-9, 11-15. All the samples collected were analyzed for target pesticides related to this study (Table 17).

Pesticides were predominantly detected in early groundwater sampling events. Pesticide detection occurred during the first and second rounds of water sampling events, June-July 2019 and August 2019, respectively. The higher concentrations of pesticides were detected in the early summer months, June-July, compared to late summer month, August. This was expected since the sampling events overlapped with Wisconsin's growing season, mid-May to early October, when pesticides are actively applied. Usually, over-the-counter lawn care products follow a multistep application process. Each application process consistently applies pesticides during the spring months, March-June, to prevent any pre-emergent pests and be

effective throughout the growing season. During the summer month, June-September, pesticides are typically for spot treatments.

During the sampling events outside of the growing season, November 2019 and February 2020, no pesticides were detected above the LOQ, which was expected. The occurrence of pesticides during the growing season and non-growing season were to be expected.

The highest concentration of pesticide detected, dicamba, appeared during the first sampling event in June-July 2019. Well 5 displayed a 2.18 ng/mL concentration of dicamba. The concentration was significantly below the Wisconsin health-based enforcement standard (ES), 300 ng/mL, and significantly below the prevention action level, 60 ng/mL. The surrogate standard, MCPA-13C6, recovery for this particular sample was 71.2%. Dicamba did not appear in any other well sample in any of this or other sampling events.

The second highest detected pesticide, MCPA, was detected in Well 2. During the June-July 2019 sampling event, MCPA was detected in Well 2 at a concentration of 0.16 ng/mL. MCPA does not have an enforcement standard, prevention action limit or a drinking water maximum contamination limit. The surrogate standard (MCPA-13C6) had a 69.4% recovery for this sample. No other sample from the sampling events displayed concentration of MCPA.

Well 13 had more than one pesticide detected in one sample during the first sampling events in June-July 2019. One of the pesticides detected was Imidacloprid at a concentration of 0.04 ng/mL. The surrogate standard (Imidacloprid-D4) recovery was 68.5% for this sample.

Table 17 Pesticide Detection Results

Round 1: June-July 2019

Well Study #	Sample Date	Concentration of 1L Sample (ng/mL)							Surrogate Recovery (%)	
		Carbaryl	Imidacloprid	Malathion	MCPA	MCPP	2,4-D	Dicamba	Imidacloprid _D4	MCPA_ 13C6
1	1-Jul	*	*	*	*	*	*	*	53.1%	70.8%
2	18-Jun	*	*	*	0.16	*	*	*	56.2%	69.4%
3	24-Jun	*	*	*	*	*	*	*	57.2%	87.2%
4	12-Jun	*	*	*	*	*	*	*	69.3%	61.2%
5	11-Jun	*	*	*	*	*	*	2.18	61.2%	71.2%
6	11-Jun	*	*	*	*	*	*	*	70.9%	69.2%
9	22-Jul	*	*	*	*	*	0.025	*	77.5%	68.5%
11	23-Jul	*	*	*	*	*	*	*	45.8%	72.3%
12	3-Jul	*	*	*	*	*	0.06	*	59.8%	58.2%
13	4-Jul	*	0.04	0.27	*	*	*	*	68.5%	56.8%
14	19-Jun	*	*	*	*	*	0.008	*	61.7%	55.2%
15	19-Jun	*	*	*	*	*	*	*	74.1%	68.2%
16	12-Jun	*	*	*	*	*	*	*	45.5%	52.3%

* = below detection limit

Table 17 (cont.) Pesticide Detection Results

Round 2: August-September 2019

Well Study #	Sample Date	Concentration of 1L Sample (ng/mL)							Surrogate Recovery (%)	
		Carbaryl	Imidacloprid	Malathion	MCPA	MCPP	2,4-D	Dicamba	Imidacloprid_D4	MCPA_13C6
1	21-Aug	*	*	*	*	*	*	*	58.5%	67.3%
2	10-Sep	*	*	*	*	*	*	*	78.8%	77.1%
3	16-Aug	*	*	*	*	*	*	*	54.6%	84.7%
4	23-Aug	*	*	*	*	*	*	*	69.8%	66.0%
5	28-Aug	*	*	*	*	*	*	*	61.7%	80.7%
6	19-Aug	*	*	*	*	*	*	*	52.7%	57.8%
7	30-Aug	*	*	*	*	*	*	*	76.8%	65.3%
8	28-Aug	*	*	*	*	*	*	*	58.1%	54.0%
9	28-Aug	*	*	*	*	0.01	*	*	79.8%	76.0%
10	27-Aug	*	*	*	*	*	*	*	60.2%	78.0%
11	27-Aug	*	*	*	*	*	*	*	67.3%	70.4%
12	26-Aug	*	*	*	*	*	*	*	52.7%	62.2%
13	19-Aug	*	*	*	*	*	*	*	68.3%	68.0%
14	29-Aug	*	*	0.032	*	*	*	*	79.0%	53.8%
15	29-Aug	*	*	*	*	*	*	*	80.5%	86.4%
16	26-Aug	*	*	*	*	*	*	*	73.4%	86.4%

* = below detection limit

Table 17 (cont.) Pesticide Detection Results

Round 3: November 2019

Well Study #	Sample Date	Concentration of 1L Sample (ng/mL)							Surrogate Recovery (%)	
		Carbaryl	Imidacloprid	Malathion	MCPA	MCPP	2,4-D	Dicamba	Imidacloprid_D4	MCPA_13C6
2	26-Nov	*	*	*	*	*	*	*	47.2%	76.0%
5	21-Nov	*	*	*	*	*	*	*	72.2%	105.7%
7	12-Nov	*	*	*	*	*	*	*	60.0%	74.6%
8	14-Nov	*	*	*	*	*	*	*	66.0%	61.6%
9	22-Nov	*	*	*	*	*	*	*	65.8%	81.4%
10	22-Nov	*	*	*	*	*	*	*	51.0%	68.9%
11	20-Nov	*	*	*	*	*	*	*	66.2%	99.7%
12	8-Nov	*	*	*	*	*	*	*	65.0%	103.6%
13	12-Nov	*	*	*	*	*	*	*	49.3%	103.6%
14	11-Nov	*	*	*	*	*	*	*	74.7%	103.0%
15	11-Nov	*	*	*	*	*	*	*	67.5%	102.1%

* = below detection limit

Table 17 (cont.) Pesticide Detection Results

Round 4: February 2020

Well Study #	Sample Date	Concentration of 1L Sample (ng/mL)							Surrogate Recovery (%)	
		Carbaryl	Imidacloprid	Malathion	MCPA	MCPP	2,4-D	Dicamba	Imidacloprid_D4	MCPA_13C6
2	14-Feb	*	*	*	*	*	*	*	76.4%	70.9%
3	14-Feb	*	*	*	*	*	*	*	96.0%	70.6%
5	6-Feb	*	*	*	*	*	*	*	71.4%	91.7%
7	2-Feb	*	*	*	*	*	*	*	80.2%	73.2%
8	6-Feb	*	*	*	*	*	*	*	86.0%	71.2%
9	12-Feb	*	*	*	*	*	*	*	75.1%	84.6%
11	10-Feb	*	*	*	*	*	*	*	61.5%	51.6%
12	3-Feb	*	*	*	*	*	*	*	69.0%	60.0%
13	4-Feb	*	*	*	*	*	*	*	70.1%	82.9%
14	3-Feb	*	*	*	*	*	*	*	38.7%	59.2%
15	3-Feb	*	*	*	*	*	*	*	54.6%	52.4%

* = below detection limit

Similar to MCPA, Imidacloprid does not have an enforcement standard, prevention action limit or a drinking water maximum contamination limit. Imidacloprid was not detected in any other sample during any other event.

The second pesticide that appeared in Well 2 during the June-July sampling event was malathion. The concentration detected in Well 2 for malathion was 0.27 ng/mL with a surrogate standard (imidacloprid-D4) recovery of 68.5%. Unlike the other pesticides, malathion was detected in more than one wells. During the second sampling event, August 2019, malathion was also detected in Well 14. Well 14's concentration for malathion was 0.032 ng/mL with a surrogate standard recovery of 79.0%. Malathion does not have an enforcement standard, prevention action limit or a drinking water maximum contamination limit either.

MCPP was detected in one well during the second sampling event in August 2019. Well 9 showed a concentration of 0.01 ng/mL with a surrogate standard recovery of 76.0%. MCPP does not have an enforcement standard, prevention action limit or a drinking water maximum contamination limit.

The most common pesticide detected was 2,4-D with three occurrences in 3 different wells. All of the wells were part of the first sampling event in June-July 2019. Well's 9, 12, 15 had a concentration of 0.025, 0.06, and 0.008 ng/mL, respectively. The surrogate standards recovery for the well samples 9, 12, and 15 where 68.5%, 58.2%, and 55.2%, respectively. None of detected concentrations exceed Wisconsin's groundwater enforcement standard, prevention action limit or drinking water maximum contamination limit for 2,4-D which are 70ng/mL, 7ng/mL and 70 ng/mL, respectively.

Overall, results showed that none of the well displayed any concentration above exciting enforcement standard, prevention action limit or drinking water MCL for Wisconsin.

CHAPTER 5: CONCLUSIONS, LIMITATIONS, AND RECOMMENDATIONS

5.1 Conclusions

This study focuses on developing, optimizing, and validating a method for simultaneous, trace detection of 7 pesticides in groundwater from Milwaukee, WI metropolitan area. The development of the method considered various option for optimization with respect to chromatography and MS conditions.

Chromatographic optimization focused on selecting appropriate mobile phase solvents, analytical column, column temperature and solvent for standard preparation. The optimization was done to achieve optimum peak profiles, resolution, and appropriate retention time.

MS optimization included a sequence of scans to produce an MRM method appropriate for analyte detection. The optimization included selecting appropriate precursor ions from a SIM scan. Since two analytes, 2,4-D and dicamba, produced a similar abundant precursor ion, the method required the precursor ion to do further fragmented into product ions for confirmation.

LLE and SPE pre-treatment processes were examined. Based on the recovery data, and due in part to time constraints, LLE was best suited for the study. Unfortunately, there were two separate LLE processes chosen, method-A and method-B, since each was appropriate for certain target analytes.

After the complete optimization method was validated, it was used to detect and quantitate target pesticide analytes in water sample collected from groundwater wells in Milwaukee, Wisconsin metropolitan area. Samples were collected during four different sampling events: June-July 2019, August 2019, November 2019, and February 2020. Out of 7

target pesticides, only 6 were detected in the groundwater samples. Luckily, none of the pesticide detected where above any existing enforcement standard, prevention action limit, or maximum contamination level currently in place. Unfortunately, 4 out of the 7 pesticides do not have a health-based enforcement standard.

With the limited data collected from the study, it was observed that groundwater is the most susceptible to pesticide contamination during the late spring and early summer months. Pesticides appeared in samples collected in the first and second sampling event, June-July and August 2019, respectively. This was not surprising since this is the time frame when homeowners and professional lawn care companies apply the most of pesticides to lawns. Additionally, groundwater recharge typically take place during this time period as well.

Of the pesticides detected, the most frequent was 2,4-D which appeared in 3 wells during the first sampling event in June-July 2019. The most abundant pesticide detected was dicamba at a concentration of 2.18 ng/mL in well 5 during the first sampling event.

5.3 Recommendations

Because of time constrains, two pre-treatment procedures were used in study. Further research is needed to combine the cleanup method for all the pesticides simultaneously by using one solvent rather than two.

One of the challenges was to improve the recovery rate for the surrogate standard for environmental samples. Environmental sample matrices varied from location to location which could have an impact on surrogate standard recovery. Further research would be required to help develop a preconcentration method with greater accuracy and reliability.

Pesticide concentrations were observed during the early spring and summer months. This would suggest that groundwater is more susceptible to contamination during this time of the year. However, a further long-term data collection and analysis is recommended to fully investigate potential seasonal variability. Although the severity and frequency of detection does not compare to those done in an agricultural setting, testing for residential pesticides should continue to be monitored for historical trends and potential health-based implications.

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APPENDIX A: PESTICIDE ANALYSIS DETAILS

Appendix A displays the procedure of qualitative analysis of the selected pesticides and surrogate standards.

Table A1 SIM Analysis

m/z	Absolute Intensity	Relative Intensity
Carbaryl		
200.9	2,419	7.58
201	1,375	4.31
201.1	3,954	12.39
201.2	3,421	10.72
201.3	4,503	14.11
201.4	8,093	25.35
201.5	31,922	100
Imidacloprid		
254.5	12	0
254.6	1,697	0.56
254.7	4,835	1.59
254.8	9,760	3.21
254.9	11,335	3.73
255	18,999	6.25
255.1	20,258	6.66
255.2	26,652	8.76
255.3	32,302	10.62
255.4	60,399	19.86
255.5	304,181	100
Malathion		
329.5	5,211	0.29
329.6	5,950	0.33
329.7	8,266	0.46
329.8	11,020	0.61
329.9	13,372	0.75
330	16,926	0.94
330.1	20,262	1.13
330.2	31,442	1.75
330.3	98,033	5.46
330.4	796,339	44.38
330.5	1,794,430	100

m/z	Absolute Intensity	Relative Intensity
Imidacloprid-D4		
259.5	245,455	2.8
259.6	778,252	8.88
259.7	1,237,688	14.12
259.8	4,001,418	45.66
259.9	8,762,676	100
260	5,238,048	59.78
260.1	8,173,894	93.28
260.2	8,667,600	98.91
260.3	5,749,446	65.61
260.4	210,185	2.4
260.5	49,836	0.57
MCPA		
198.5	1,367	1.13
198.6	11,195	9.25
198.7	33,133	27.38
198.8	41,144	34
198.9	71,601	59.17
199	80,065	66.16
199.1	79,134	65.39
199.2	121,014	100
199.3	81,920	67.69
199.4	38,230	31.59
199.5	3,282	2.71
MCPP		
212.5	2,180	1.18
212.6	23,374	12.61
212.7	49,007	26.44
212.8	61,018	32.92
212.9	120,503	65.02
213	115,827	62.5
213.1	126,525	68.27
213.2	185,331	100
213.3	120,661	65.11
213.4	55,580	29.99
213.5	4,874	2.63

Table A1 (cont.) SIM Analysis

m/z	Absolute Intensity	Relative Intensity
2,4-D		
218.5	14,734	7.18
218.6	52,931	25.79
218.7	64,260	31.3
218.8	99,379	48.41
218.9	146,535	71.39
219	122,168	59.52
219.1	205,271	100
219.2	113,783	55.43
219.3	91,285	44.47
219.4	23,239	11.32
219.5	2,380	1.16
Dicamba		
218.5	775	12.17
218.6	1,640	25.76
218.7	2,347	36.87
218.8	3,560	55.92
218.9	4,608	72.38
219	4,462	70.09
219.1	6,366	100
219.2	4,161	65.36
219.3	2,930	46.03
219.4	1,036	16.27
219.5	241	3.79
MCPA-13C6		
204.5	54,040	47.25
204.6	86,196	75.36
204.7	83,616	73.1
204.8	102,631	89.73
204.9	94,556	82.67
205	114,380	100
205.1	65,594	57.35
205.2	66,433	58.08
205.3	28,492	24.91
205.4	11,043	9.65
205.5	11,084	9.69

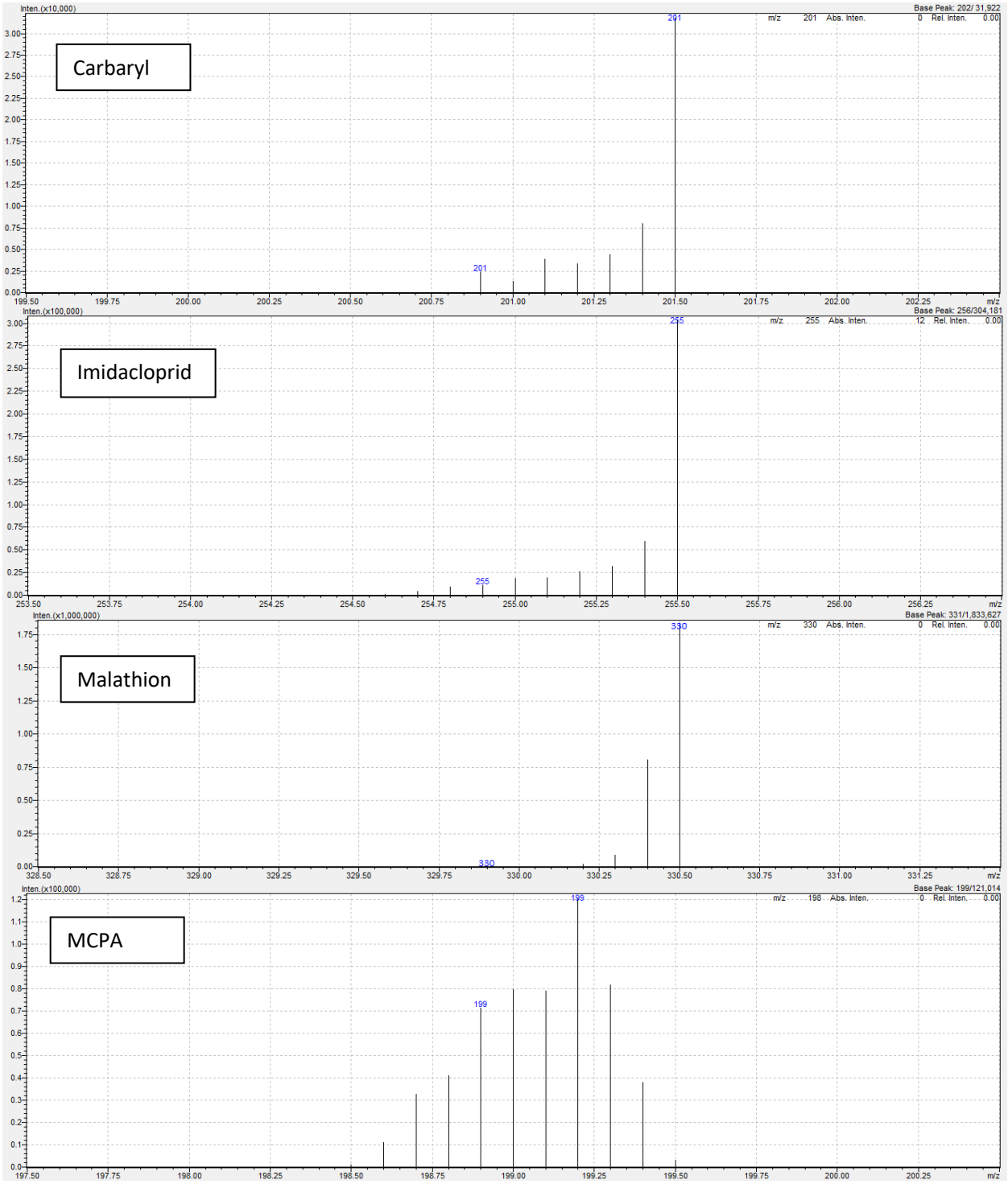

Figure A1 SIM Scan Mass Spectrum (m/z vs. Absolute Intensity)

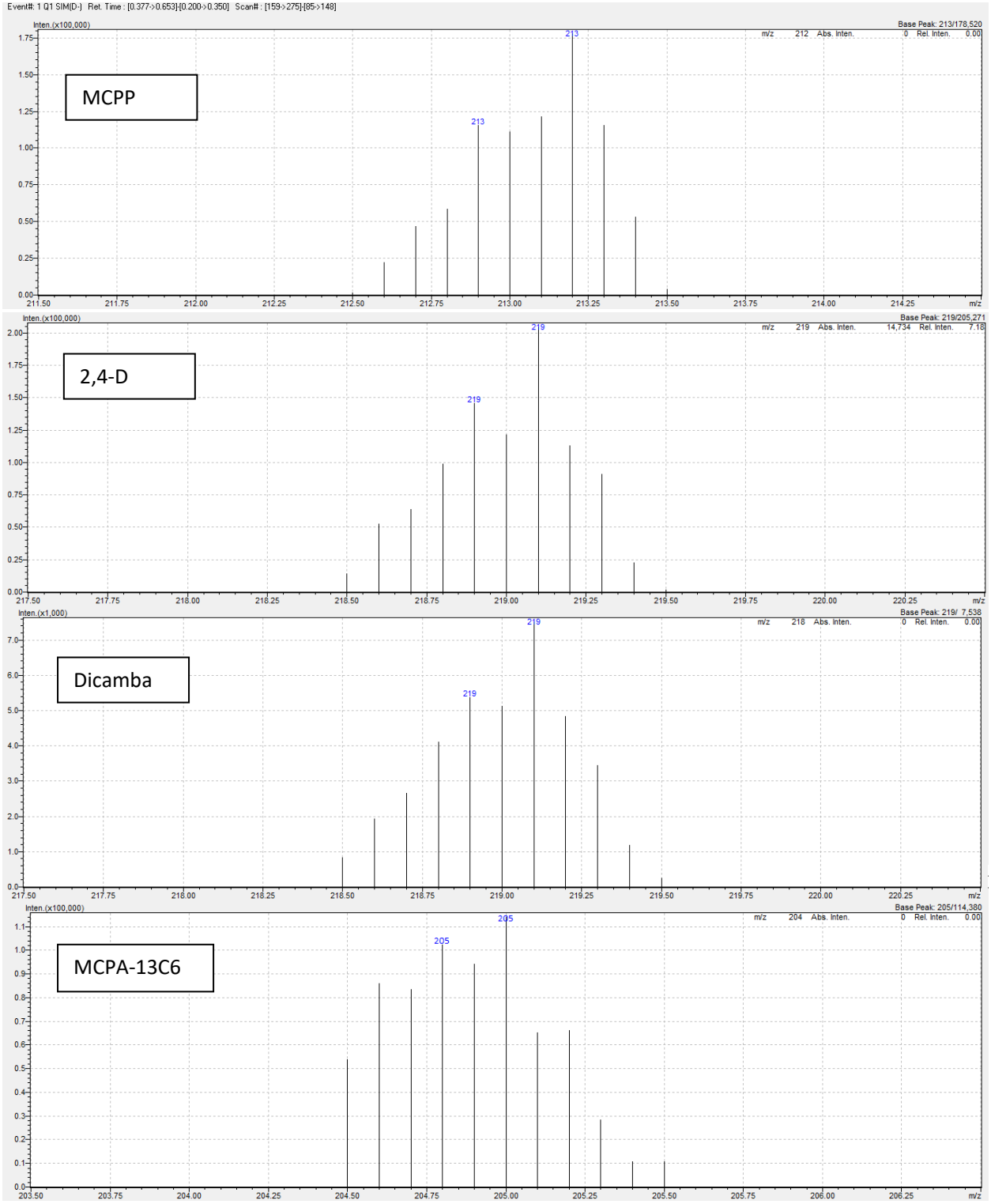

Figure A1(cont.) SIM Scan Mass Spectrum (m/z vs. Absolute Intensity)

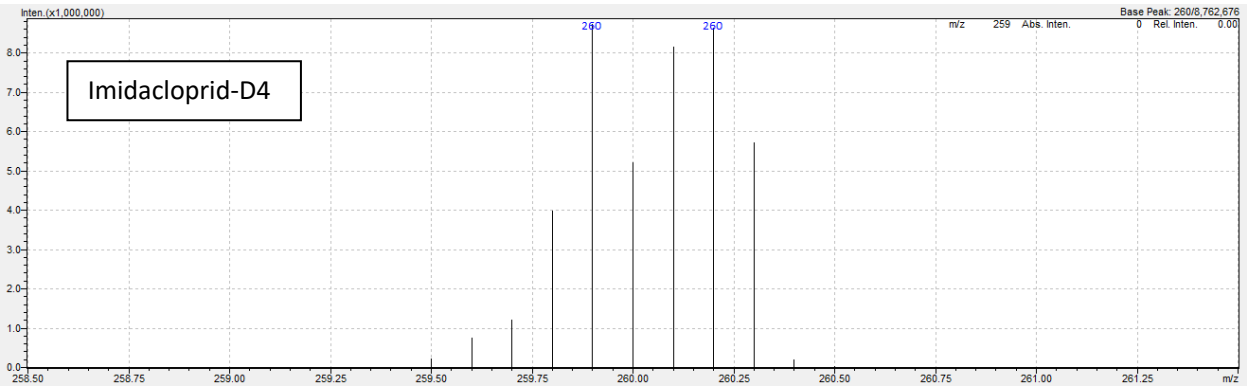

Figure A1 (cont.) SIM Scan Mass Spectrum (m/z vs. Absolute Intensity)

Table A2 PI analysis for positive ions

m/z	Absolute Intensity (CE = -10V)	Absolute Intensity (CE = -15V)	Absolute Intensity (CE = -20V)	Absolute Intensity (CE = -25V)	Absolute Intensity (CE = -30V)	Absolute Intensity (CE = -35V)	Absolute Intensity (CE = -40V)	Absolute Intensity (CE = -45V)	Absolute Intensity (CE = -50V)
Carbaryl									
145.0	12,958	9,075	5,725	3,272	1,173	679	268	473	*
76.0	*	*	473	*	*	*	851	2,056	1,389
19.0	*	*	947	*	*	*	*	*	*
Imidacloprid									
209.0	32,800	46,350	39,222	27,401	15,326	10,857	4,302	6,486	1,085
175.0	26,372	33,491	43,209	31,938	16,280	7,951	2,906	695	2,127
118.0	2,603	4,522	*	2,694	2,038	777	1,922	1,486	1,544
Malathion									
285.0	407,782	55,083	*	*	*	*	*	*	*
127.0	433,567	529,697	221,122	85,502	391,687	*	*	*	*
99.0	30,733	169,971	355,411	445,201	26,695	275,054	194,483	133,452	86,428
Imidacloprid-D4									
214.0	409,201	271,592	92,313						
213.0	1,209,369	1,438,122	1,154,713	926,398	658,000	433,328	225,206	107,867	53,025
179.0	766,670	1,300,658	1,359,382	1,049,655	678,188	337,866	144,389	72,003	32,359

note: * = not data found

Table A3 PI analysis for negative ions

m/z	Absolute Intensity (CE = 10V)	Absolute Intensity (CE = 15V)	Absolute Intensity (CE = 20V)	Absolute Intensity (CE = 25V)	Absolute Intensity (CE = 30V)	Absolute Intensity (CE = 35V)	Absolute Intensity (CE = 40V)	Absolute Intensity (CE = 45V)	Absolute Intensity (CE = 50V)
MCPA									
141.1	184,181	188,771	129,666	97,295	31,701	16,048	5,722	3,590	2,908
105.0	*	*	*	*	5,202	1,501	1,047	476	1,196
35.0	*	*	*	*	7,582	7,033	7,343	8,452	4,649
MCPP									
141.0	271,181	264,590	167,357	119,625	58,660	33,410	10,790	4,250	3,564
105.0	*	*	*	*	6,840	2,103	*	2,582	659
35.0	*	*	*	*	7,525	7,536	9,669	7,720	3,997
2,4-D									
161.0	273,567	271,697	188,251	109,841	42,682	15,177	13,004	5,201	1,922
125.0			19,389	23,510	20,963	16,283	3,184	4,071	1,139
35.0				6,203	7,500	7,576	5,775	7,307	6,509
Dicamba									
175.0	80,137	14,421	1,693	1,121	727	709	*	*	524
35.0	6,363	7,038	5,447	7,774	7,628	5,807	4,058	4,885	3,793
MCPA-13C6									
35.0				5,465	8,099	9,084	3,475	8,589	5,167
147.0	165,655	218,022	161,936	108,034	54,028	21,175	9,620	4,831	4,094

note: * = not data found

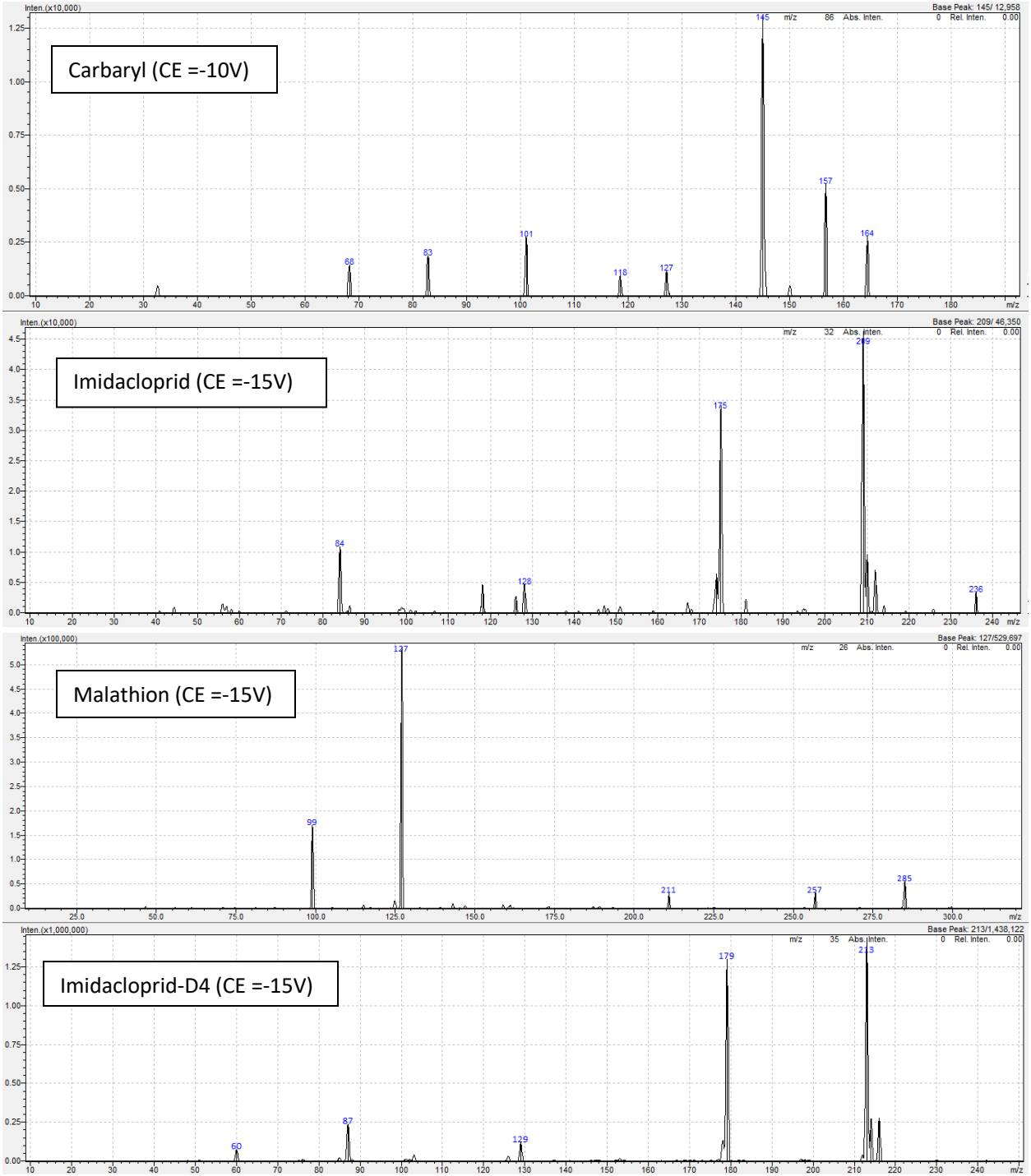

Figure A2 PI analysis scan mass spectrum for positive ions. (m/z vs Absolute Intensity)

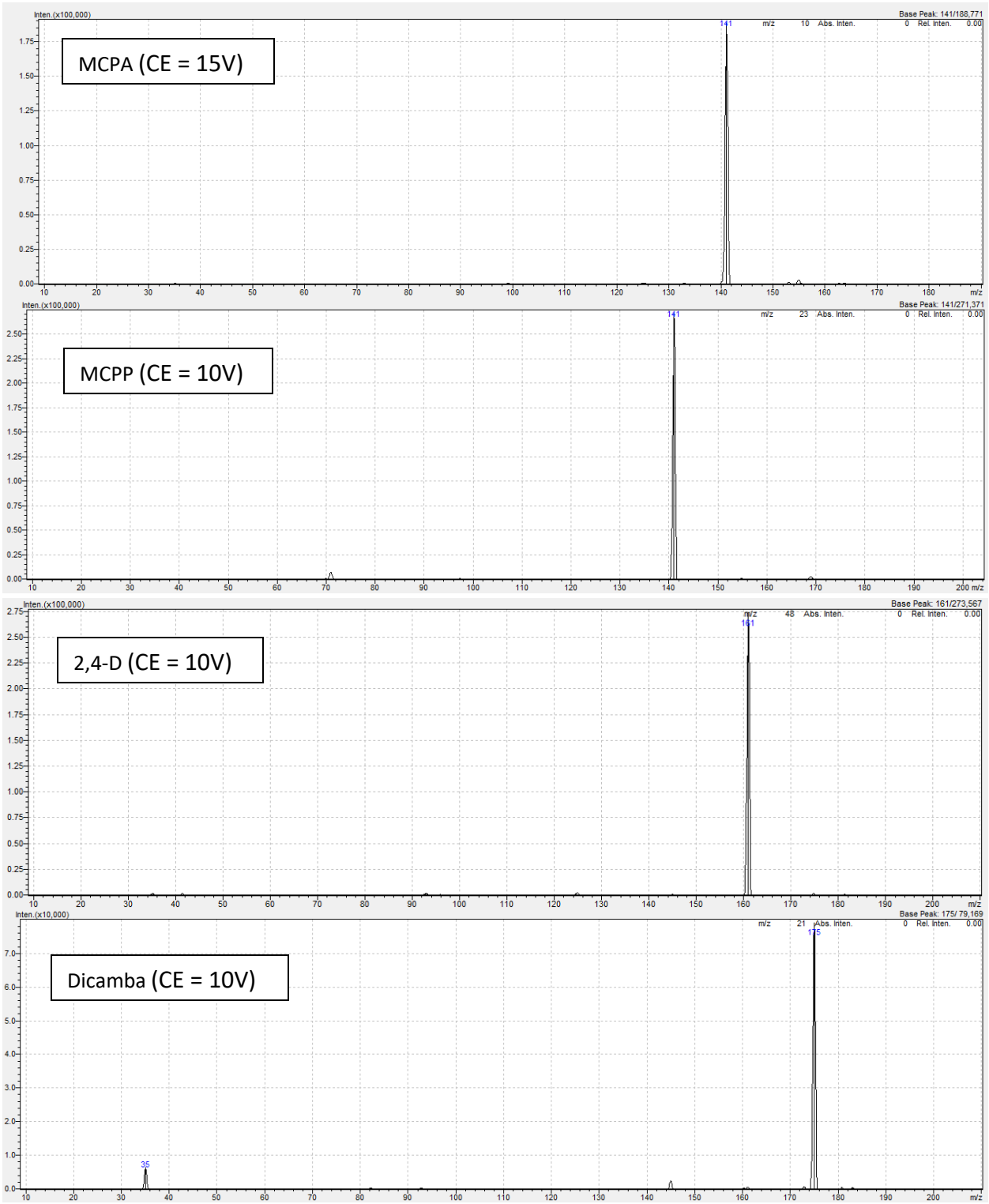

Figure A3 PI analysis scan mass spectrum for negative ions (m/z vs Absolute Intensity)

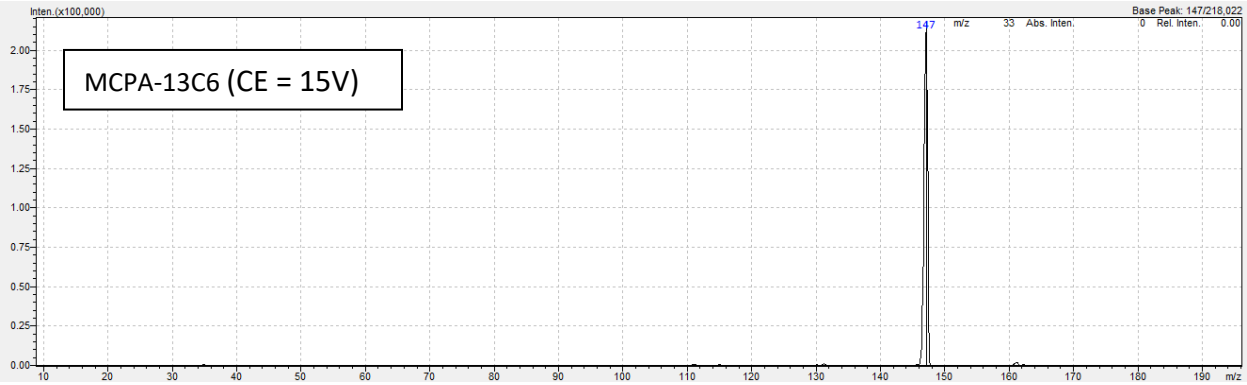

Figure A3(cont.) PI analysis scan mass spectrum for negative ions (m/z vs Absolute Intensity)

Table A4 Optimization of PI analysis for negative ions

m/z	Absolute Intensity	Relative Intensity
Carbaryl (CE = -11V)		
144.5	456	4.21
144.6	1803	16.66
144.7	5550	51.27
144.8	7671	70.87
144.9	9730	89.89
145	10824	100
145.1	10584	97.78
145.2	10510	97.1
145.3	8349	77.13
145.4	986	9.11
145.5	80	0.74
Imidacloprid (CE = -14V)		
208.6	6946	12.48
208.7	26221	47.1
208.8	38152	68.53
208.9	47528	85.37
209	55672	100
209.1	55014	98.82
209.2	51730	92.92
209.3	41016	73.67
209.4	8355	15.01
209.5	1373	2.47
209.6	1716	3.08
Malathion (CE = -13V)		
126.55	52158	8.63
126.65	221632	36.65
126.75	385286	63.71
126.85	476342	78.77
126.95	559614	92.54
127.05	604714	100
127.15	580084	95.93
127.25	502222	83.05
127.35	204009	33.74
127.45	14818	2.45
127.55	2304	0.38

Imidacloprid-D4 (CE = -15V)		
212.5	239521	11.7
212.6	677918	33.11
212.7	1194679	58.34
212.8	1648922	80.53
212.9	1959617	95.7
213	2047707	100
213.1	1909521	93.25
213.2	1762033	86.05
213.3	955196	46.65
213.4	98911	4.83
213.5	58059	2.84

Table A5 Optimization of PI analysis for Positive Ions

m/z	Absolute Intensity	Relative Intensity
MCPA (CE = 12V)		
140.6	67238	25.52
140.7	126846	48.14
140.8	158233	60.05
140.9	203339	77.17
141	243439	92.38
141.1	263507	100
141.2	236967	89.93
141.3	84222	31.96
141.4	9918	3.76
141.5	1993	0.76
141.6	656	0.25
MCPP (CE = 13V)		
140.5	37514	10.49
140.6	92845	25.96
140.7	175100	48.95
140.8	218961	61.22
140.9	278636	77.9
141	333627	93.28
141.1	357678	100
141.2	327970	91.69
141.3	116551	32.59
141.4	13380	3.74
141.5	2715	0.76

Table A5 (cont.) Optimization of PI analysis for Positive Ions

2,4-D (CE = 12V)		
160.5	63791	18.5
160.6	136300	39.52
160.7	186834	54.17
160.8	249531	72.35
160.9	305107	88.47
161	344876	100
161.1	335249	97.21
161.2	232240	67.34
161.3	33190	9.62
161.4	5350	1.55
161.5	1457	0.42
Dicamba (CE = 8V)		
174.5	15983	14.77
174.6	35181	32.51
174.7	55909	51.66
174.8	72987	67.44
174.9	90661	83.77
175	105384	97.37
175.1	108232	100
175.2	88868	82.11
175.3	20195	18.66
175.4	2649	2.45
175.5	513	0.47
MCPA-13C6 (CE = 13V)		
146.6	39561	20.81
146.7	59661	31.39
146.8	107807	56.71
146.9	147256	77.47
147	176319	92.76
147.1	190086	100
147.2	172440	90.72
147.3	52066	27.39
147.4	6428	3.38
147.5	1638	0.86
147.6	494	0.26

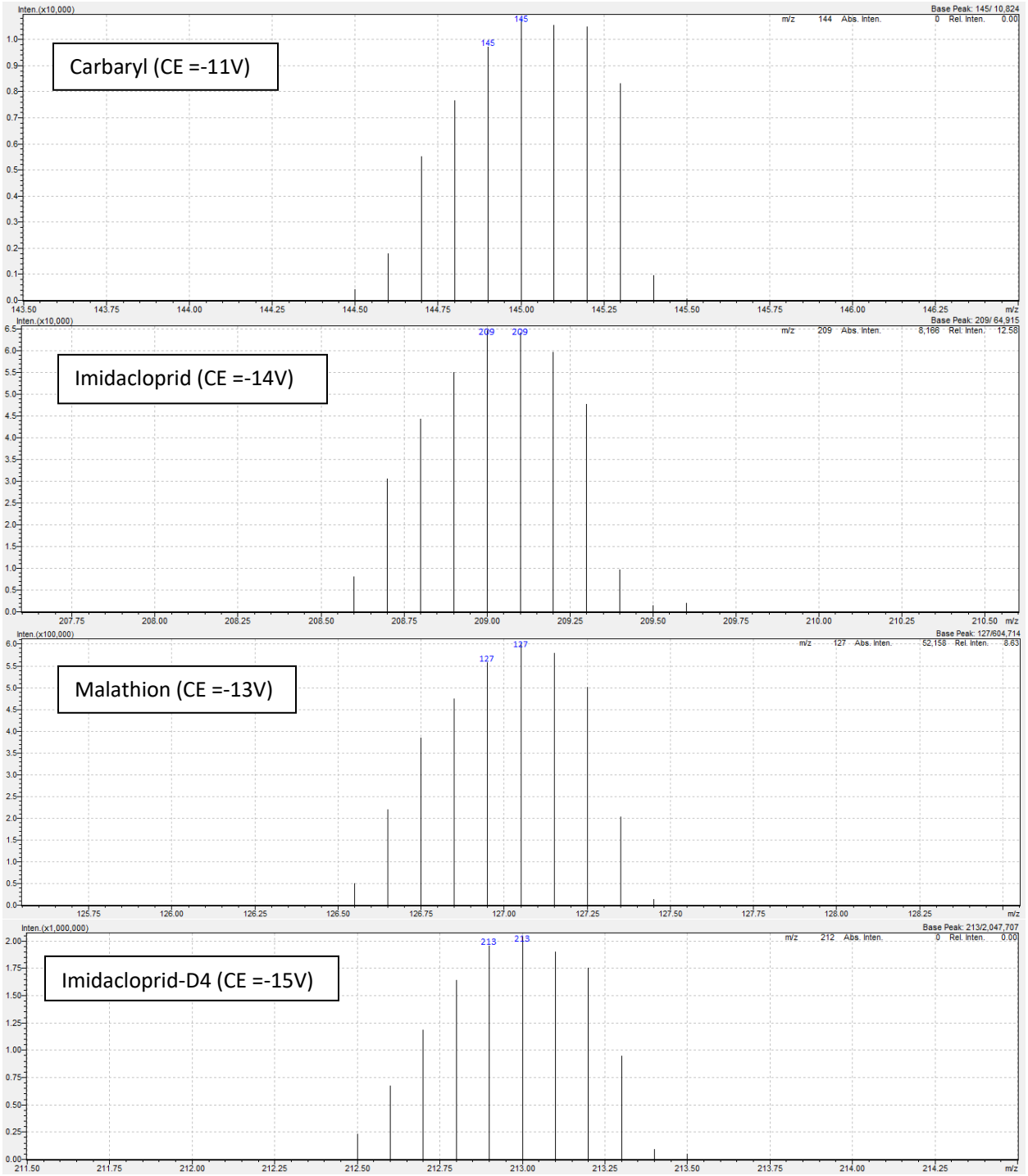

Figure A4 Optimization PI scan mass spectrum for Negative Ions (m/z vs. absolute intensity)

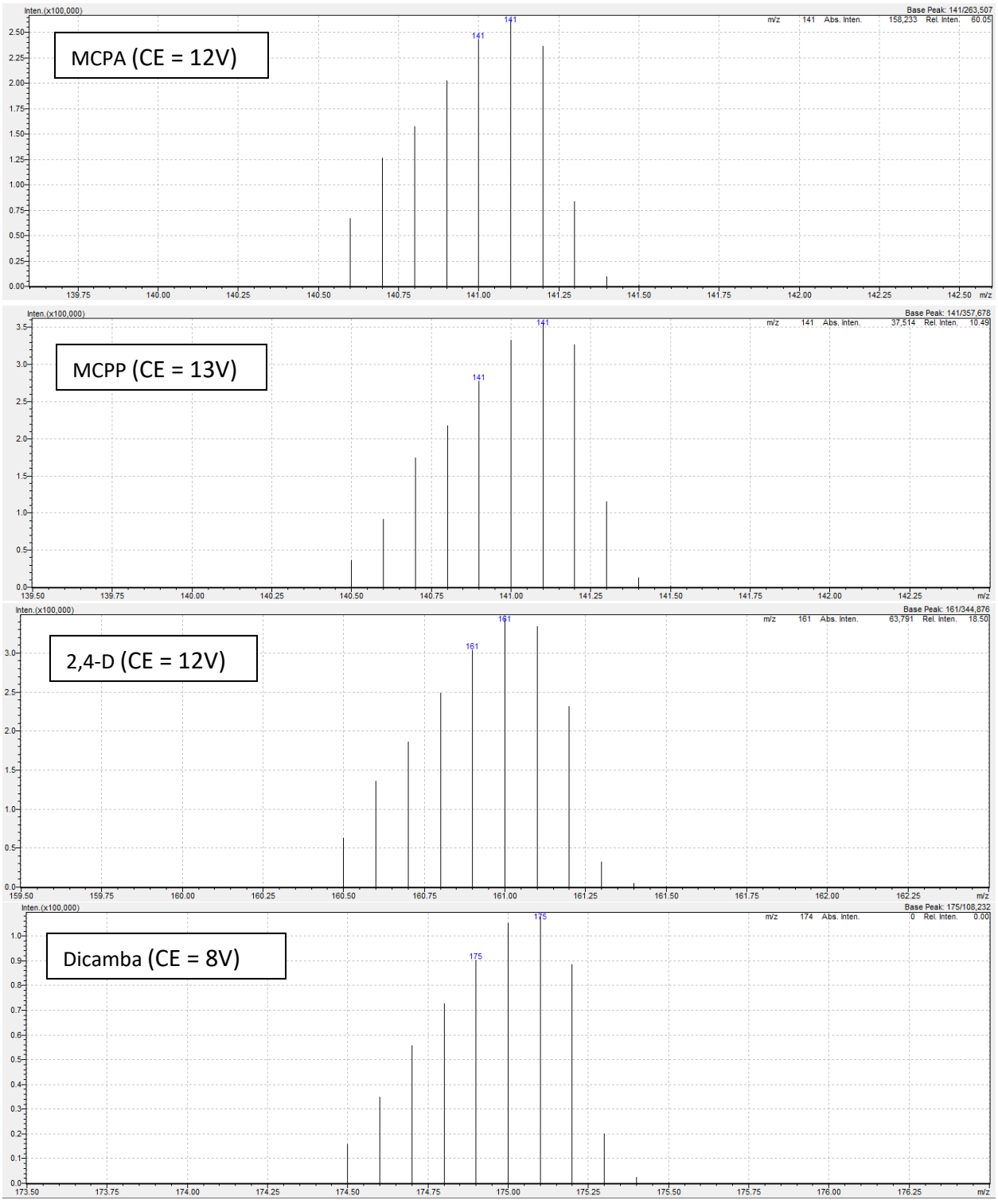

Figure A5 Optimization PI scan mass spectrum for Positive Ions (m/z vs. absolute intensity)

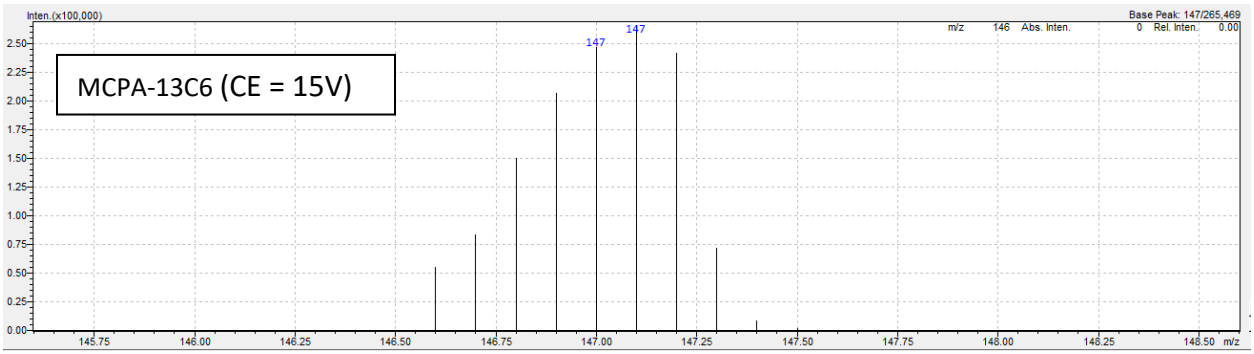

Figure A5 (cont.) Optimization PI scan mass spectrum for Positive Ions (m/z vs. absolute intensity)