Development of precise, sensitive, and simplified method for herbicide detection in Delta Water using SPE and GC-MS-MS method

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ABSTRACT

Freshwater is crucial for the survival of human society and aquatic organisms, however, the increased application of herbicides for controlling unwanted growth of plants in surface water and crop land enhances the herbicide residues in ambient water bodies such as Sacramento-San Joaquin Delta. These elevated levels of herbicide residues in water column impacts both the health of human and aquatic organisms, and controlling the residue levels requires monitoring of these residues in environmental water samples. In order to monitor herbicide residues in ambient water, this study developed a precise and sensitive method using solid phase extraction (SPE) gas chromatography (GC) and mass spectrometry (MS) for determining the low levels of herbicides in the delta water. This paper presents the results of four herbicides: 1) Fluridone; 2) Atrazine; 3) Lenacil; and 4) Triadimefon. This method is relatively a simpler and robust and potentially can be used for the water testing of any ambient fresh water bodies. The calibration curves for each compound were developed for each herbicides and R2 values were 0.99. The basic working principles of other testing methods such as ELISA and LC-MS-MS are presented. This research was crucial in terms of determining the safe application rate of herbicides in delta water, and its persistence for reducing the herbicide risks to endangered species such as Delta Smelt, which now nearly extinct. The method and approach developed here can be utilized for other ambient water bodies such as lakes and rivers, where the low level of herbicide detection is needed.

Keywords: *ambient water; herbicides and pesticide; contamination; monitoring; gas-chromatography; software*

1. INTRODUCTION

To analyze multiple herbicides, fungicides, and pesticides simultaneously, Gas Chromatography-Mass Spectrometry (GC-MS) is considered to be a suitable choice because of simplicity in analyzing the samples and it also provides the sensitivity required for environmental samples such as ambient water and food samples (Fig. 1). Analytical results of more than 200 pesticide residues (i.e., organochlorine, and organonitrogen) using GC-MS provided method limit of detection (MLOD) and Method Limit of Quantification (MLOQ) of 0.45-6.33 µg kg−1, 1.44–9.59 µg kg−1 respectively (Ahire et al. 2024, Lin et al. 2007), which is a satisfactory detection range needed for food and water analysis. In addition, to GC-MS,

other methods such as an Enzyme-Linked ImmunoSorbent Assay (ELISA) technique is also used to detect and quantify herbicides and pesticides in water, soil and food samples (Alhajj et al. 2023, Knopp et al. 1999), and it can detect analyte concentrations lower than 1-10 ng/mL. Further, liquid chromatography tandem mass spectrometry (LC-MS) is also an efficient analytical tool for detecting polar and thermally-labile pesticides and herbicides in water and food samples, and it can detect pesticide and herbicides lower than 100 ng/L (Hooper et al. 2022, Sang et al. 2022).

While all these three methods (i.e, ELISA, GC-MS, and LC-MS) (shown in Figure 1) are useful for analyzing herbicides and pesticides in water, the major difference is in the detection mechanisms used by these methods. For example, ELISA is a type of immunoassay, which is used to quantify levels of a specific analyte (pesticide/herbicide) in water and food samples (Cao et al. 2005, Knopp et al. 1999, Watts et al. 1997). Both direct ELISA and indirect ELISA uses antibody-antigen interactions for detecting the analytes. In a direct ELISA, an antigen is trapped directly on the plate, and a conjugated detection is performed. However, indirect ELISA uses an additional amplification step, and detection is a two-step process, which includes binding of unlabeled primary antibody to the specific antigen, and the use of an enzyme conjugated secondary antibody that is channeled against the primary antibody (Fig. 1) (Octobre et al. 2024, Watts et al. 1997). In LC-MS based method, which is highly selective, the sample is passed through the high pressure liquid chromatography (HPLC) column where the analytes are separated based on the interactions with the stationary phase of the column and mobile phase. Subsequently, the eluent from HPLC column is injected into a MS, where samples get ionized and analyzed using mass detector (Hooper et al. 2022). Often, HPLC is connected to MS, when identification and quantifications are needed at low levels in environmental and biological samples (Kulyyassov et al. 2021). Fragments of analytes using multiple reaction monitoring (MRM) and selected reaction monitoring (SRM) are detected, which facilitates the selection of an ion of a particular mass in the first stage of mass spectrometer, and subsequently ion products are detected in the second stage. In general, SRM and MRM is performed on triple quadrupole MS instruments that allows fragmentation to enhance selectivity (Li et al. 2020, Pires et al. 2023, Siwy et al. 2011, Ying et al. 2023) (Fig. 1).

Recently, GC-MS became one of the most important analytical method particularly for analyzing organic chemicals at low levels (Hernandez et al. 2013, Hubschmann 2015, Kim et al. 2024, Lin et al. 2007). The integration of GC (for separation) and MS (for detection) provides the most meaningful data, and it provides higher selectivity and accurate mass detection for environmental and biological samples (Hubschmann 2015). The limitation of GC-MS is that analytes needs to be volatile enough to be analyzed by GC-MS (Hernandez et al. 2013). In the past few years, GC-MS/MS (triple quadrupole i.e., QqQ) became a powerful techniques for the detection of pesticide residues in environmental samples due to the robustness, sensitivity, and selectivity (Hernandez et al. 2013). Currently, pesticide residue analysis of food and environmental samples has formed a crucial specialized field of analytical chemistry for ensuring the food and environmental safety, and the GC-MSMS methods are able to provide reliable and sensitive information at the low limits of quantification (LOQ), which is needed by the legislation (Hernandez et al. 2013).

Both GC-MS and LC-MS methods requires the application of sample preparation techniques such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE). GC-MS methods

requires that analyte of interest needs to be in non-polar solvent, while LC-MS prefers the compounds to be in polar solvent.

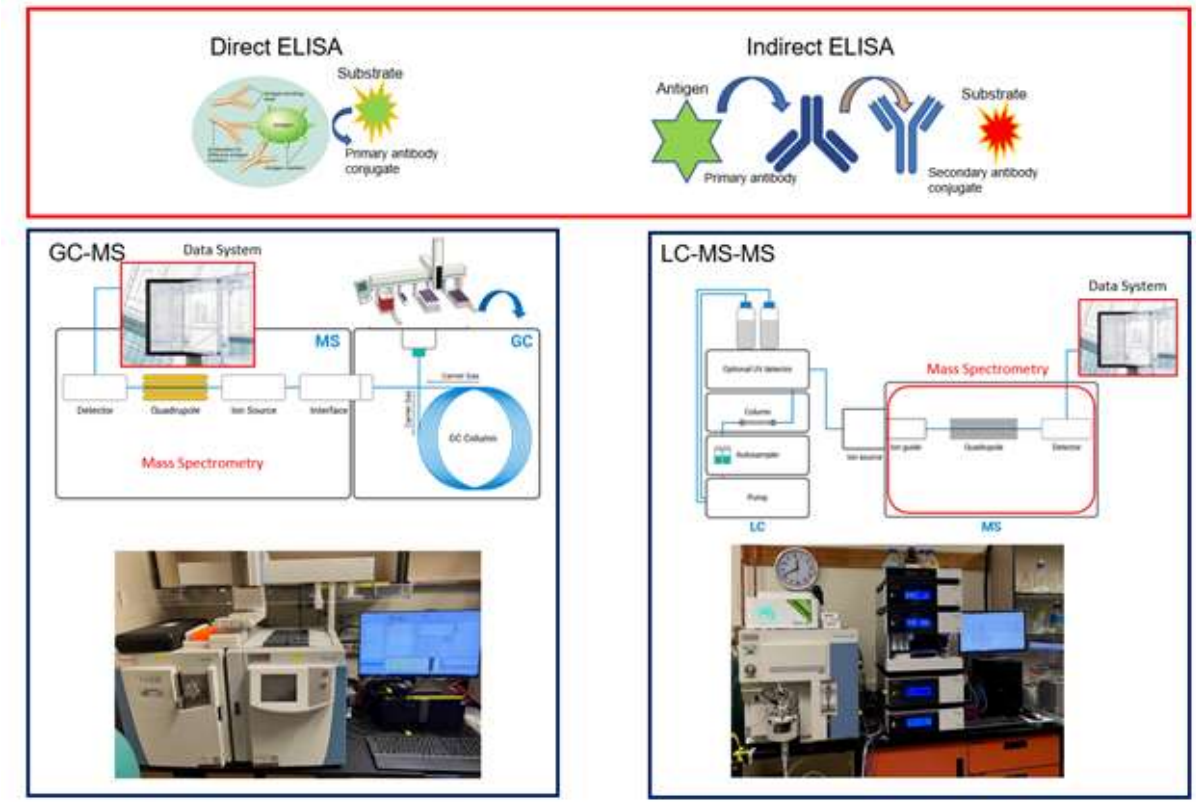


Figure 1. Working principles of Enzyme-Linked ImmunoSorbent Assay (ELISA) technique, Gas Chromatography Mass Spectrometry (GC-MS) method, and Liquid-Chromatography Mass Spectrometry (LC-MS) method.

In LLE, analytes are extracted from a sample matrix into an organic solvent based on the polarity differences, and SPE methods uses a solid-sorbent to retain analytes from a sample matrix, and subsequently suitable solvent is used to elute the analytes from the solid-sorbent. In LLE, a two-phase system is used in separation process for extraction and purification of desired molecules (Mazzola et al. 2008). In general, solid-phase extraction using cartridge is a widely used sample preparation technique for the separation, cleanup, and re-constitution of analytes (Poole 2003). Though the SPE is a time consuming process, currently SPE became a preferred method for sample preparation of target compounds from biological, environmental and food matrix (Buszewski and Szultka 2012).

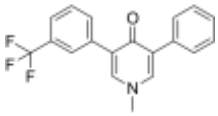
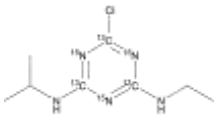
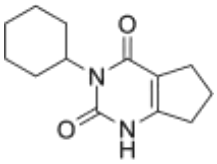
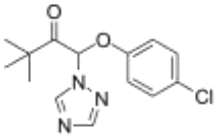
The overall goal of this research was to develop a solid phase extraction method using a cartridge for isolation, concentrations, clean-up of environmental water samples. In addition, understanding of the sample matrix and extraction techniques were used to develop and optimize GC-MS technique, which requires optimizing GC-parameters, column selection, and creating calibration curves for each analyte. The method was implemented on the water

samples collected from the Sacramento-San Joaquin River Delta. In the Delta, herbicides are used to control floating aquatic vegetation such as water hyacinth (Fig. 2). The developed method was used for the detection of four analytes: 1) Fluridone; 2) Atrazine; 3) Lenacil; and 4) Triadimefon.

Figure 2. Water hyacinth uncontrolled growth in the Sacramento-San Joaquin Delta (data source: Division of Boating and Waterways, California State Parks, CA).

The structure, molecular information, polarity, and fragmentation patterns of these compounds are shown in Table 1. Fluridone is a herbicide and it is mainly used for controlling unwanted aquatic plants (Alder et al. 2006, Huang et al. 2024). Atrazine herbicide/pesticide is used to control broad leaf and grass weeds. Lenacil herbicide is used to control unwanted grasses in many crops including sugarcane and apple crops. Triadimefon is fungicide, and it is used to prevent fungal pests on grains and fruits (Table 1).

Table 1. Herbicide and fungicide structure and application and GC-MS and LC-MS methods for detection.

Herbicides	**Structure**	**Applications**	**GC-EI-MS (m/z)**	**LC-MS-MS (m/z - m/z)**
Fluridone	 Formula: $C_{19}H_{14}F_3NO$ MW=329.32	Fluridone is a herbicide. It is mainly used to eliminate unwanted aquatic plant growth. It is a biosynthesis inhibitor. It can be used directly on the water body. It is absorbed by plants through roots and leaves and disrupts photosynthesis.	Fragment ions: 330; 329; 328	Polarity: positive 330 – 310 330 – 259
Atrazine	 Formula: $C_8H_{14}ClN_5$ MW=215.69	Atrazine is a herbicide and used to control broad leaf and grass weeds. Atrazine is absorbed by the roots and leaves of plants. It disrupt the photosynthesis process of the plant. It can also acts in the shoots of plants.	Fragment ions: 215; 200; 173	Polarity: positive 216 – 174 216 – 104
Lenacil	 Formula: $C_{13}H_{18}N_2O_2$ MW=234.29	Lenacil is a herbicide and often used to control grasses, broad leaved weeds. It is applied for weed control in crops such as sugarcane, apples, and peacans. It is absorbed through the roots and acts by inhibiting photosynthesis.	Fragment ions: 154; 153; 136	Polarity: positive 235 – 153 235 – 136
Triadimefon	 Formula: $C_{14}H_{16}ClN_3O_2$ MW=293.75	Triadimefon is a fungicide and often used to prevent fungal pests on grains, fruit, and vegetables. It interfere with oxidative demethylation reactions of fungi and blocks biosynthesis.	Fragment ions: 208; 128; 85	Polarity: positive 294 – 197 294 – 225

2. MATERIAL AND METHODS

This method used the solid phase extraction method shown in Figure 3 for sample preparation prior to the injection into GC-MS. The method requires conditioning, sample addition, washing, and elution steps. The SPE setup and extraction process is shown in Figure 3. During SPE procedure, we used OASIS Extraction Cartridge. This cartridge covers all key properties of the sorbent and assembled cartridges. Its specific surface area, average pore diameter, total pore volume, average particle diameter and fines content are determined using multipoint sorption and high-resolution and electrozone-sensing particle size analyzer. OASIS is recommended for SPE-LC-MS/MS because of its advantages of: 1) fully water-wettable sorbents increase reproducibility; 2) optimal sensitivity for small molecule; and 3) unsurpassed selectivity.

To detect the analytes of interest, we used Thermo TSQ 8000 GC-MSMS system. The TSQ 8000 offers one of the easiest way to achieve the lower detection limits. It provides reduced sample preparation allow analysis using the SRM. The TSQ 8000 is the first triple quadrupole GC-MS/MS system to offer an extractable ion source under vacuum and software. The mass spectrometer was paired with the Thermo Scientific TRACE 1310 GC (Fig. 1), which offers the unique flexibility of instant connect injector and detector modularity. We used Trace^{TM} GC columns for pesticides (catalog number 26RC142F). These columns are often used in the determination of organophosphate and organochlorine pesticides with high performance, sensitivity, and reproducibility. These columns are specifically designed and tested to meet the requirements for the analysis pesticides and herbicides and provide reproducible results. The inner diameter of the column was 0.25 mm, and stationary phase was Trace^{TM} pesticide III. It can tolerate temperature of 280-300 ^{0}C, and it has particle size of 0.2 µm, and film thickness of 0.25 µm. The length of the column was 30 m (30 m x 0.25 µm x 0.25 µm with 5 m Guard). We used helium as carrier gas, and initial oven temperature was set to 80 ^{0}C. Then temperature was increased to 300 ^{0}C at a ramp of 15 ^{0}C. The injection volume was 1.5 µL, and the total run time was 20 minutes. All required standards and solvents were purchased from Thermo Fisher Scientific, USA.

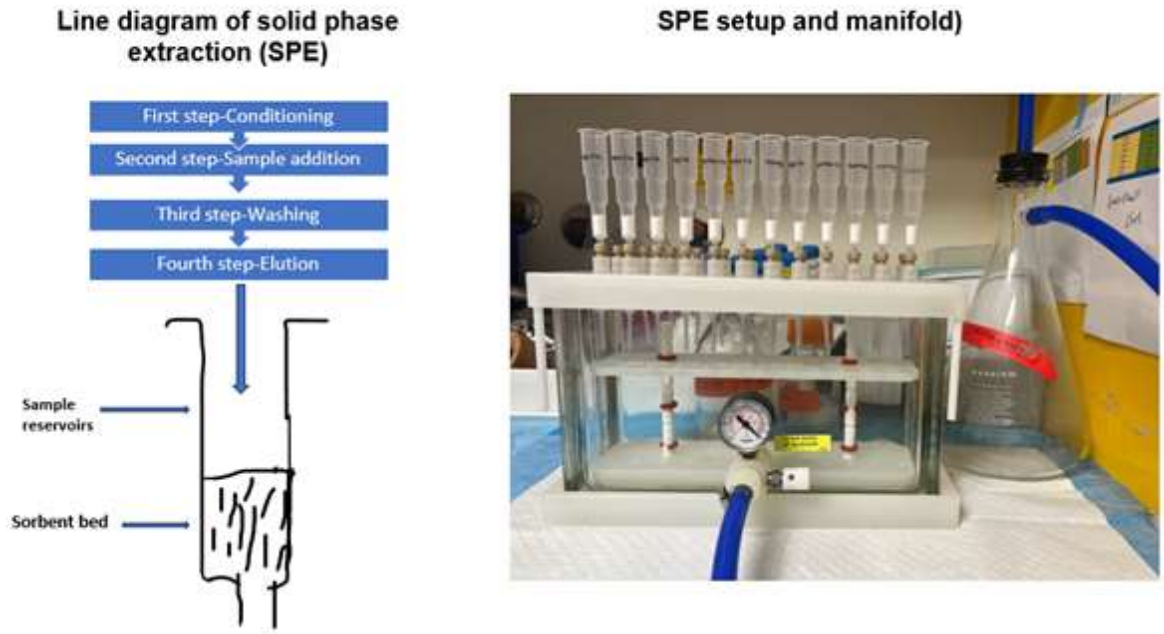


Figure 3. Solid phase extraction (SPE) procedure and setup for analyte extraction from water.

3. RESULTS AND DISCUSSION

To analyze the results and process the data, we used thermos scientific chromeleon chromatography data system (CDS) software, and the integration of peaks using CDS is shown in Figure 4. Spectra and chromatograph shown in Figure 4 is the results of Triadimefon. The integration of peak was performed using CDS, and peak areas of each level of Triadimefon was estimated and subsequently the linear relationship between areas and concentrations of Triadimefon was determined. This linear curve provided a calibration curve of Triadimefon. The similar process was adopted for each analyte of interest such as Fluridone, and CDS for

Triadimeforn is shown in Figure 5. The calibration curve for Atrazine, and Lenacil is shown in Figure 6.

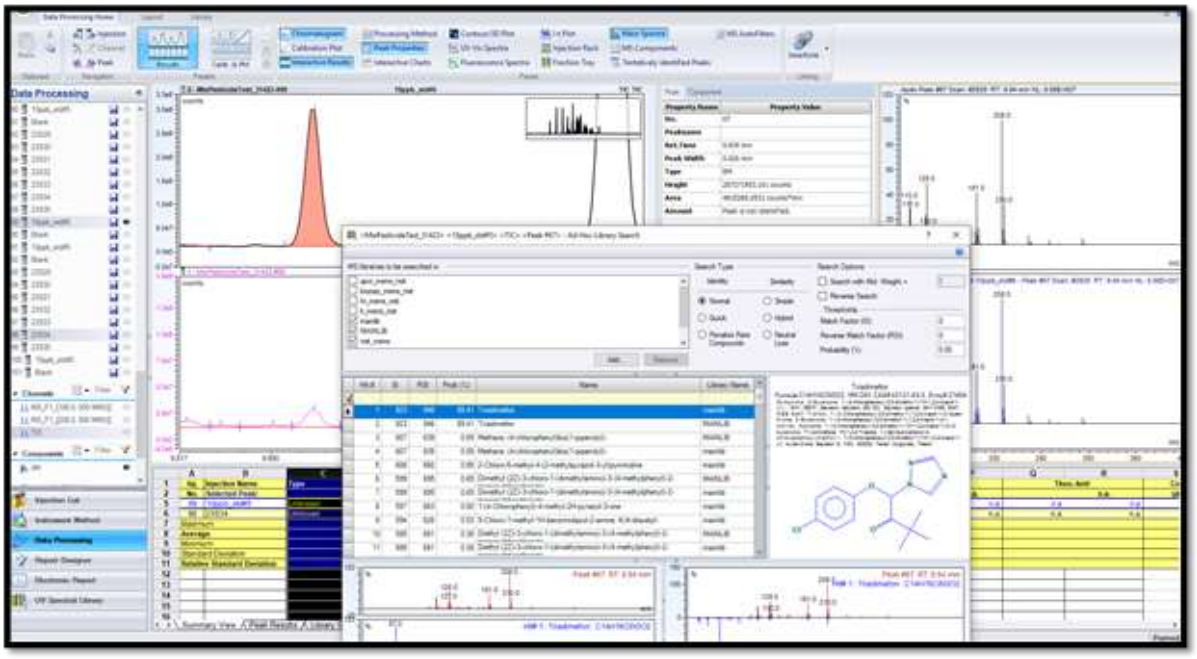


Figure 4. Thermo chromeleon data system and spectra and chromatograph of Triadimefon. The above peak shows the peak are of Triadimefon. The right side is spectra. The center window shows the library match of spectra of Triadimefon.

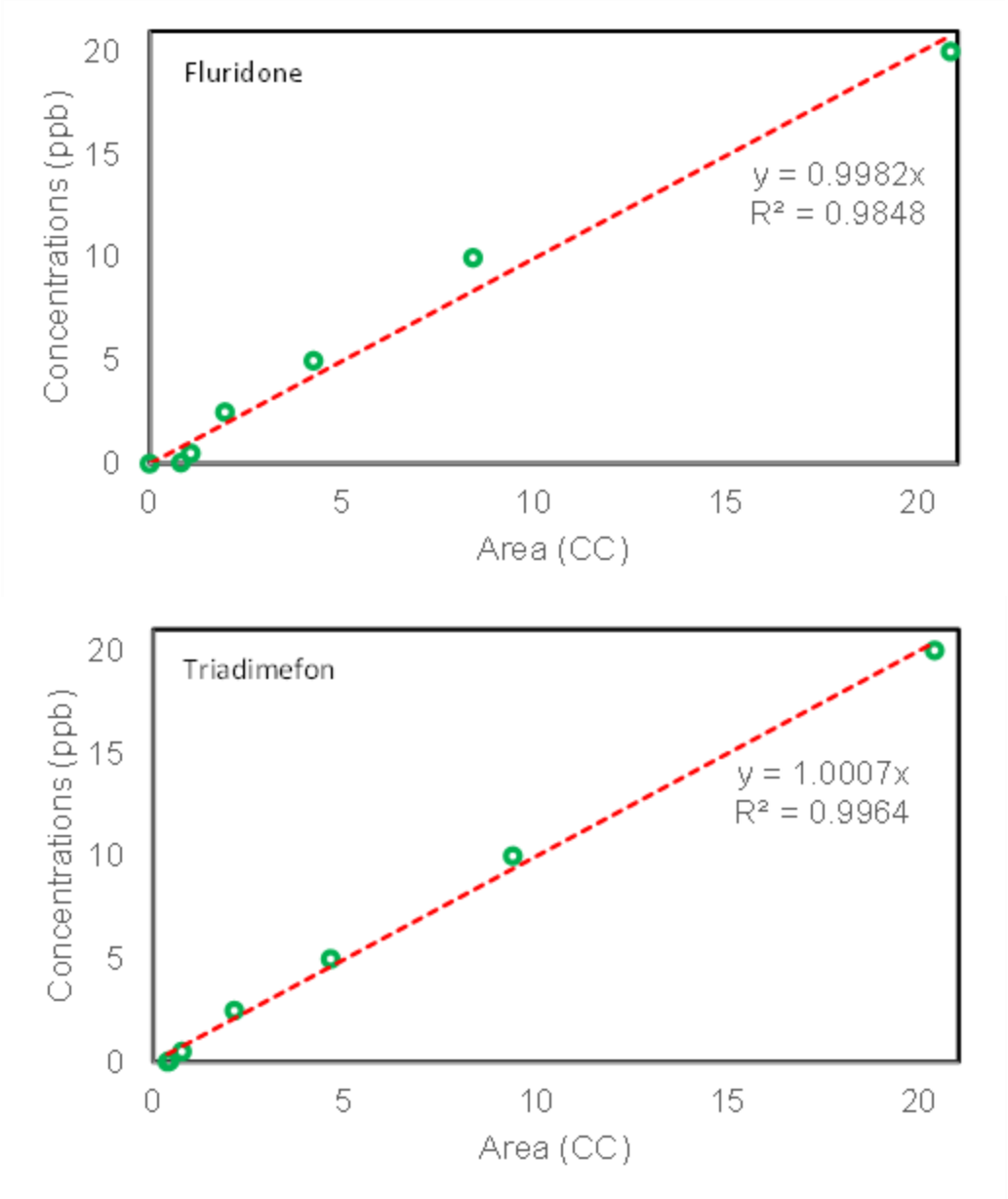


Figure 5. The calibration curves of Fluridone and Triadimefon. Top linear curve shows the linear relationships between concentrations of Fluridone and corresponding GC-MS-MS peak area of Fluridone. Bottom linear curve shows the linear relationships between concentrations

of Tri and corresponding GC-MS-MS peak area of Fluridone. The linear fit with R2 values of greater than 0.99 shows the strong relationships and successful method.

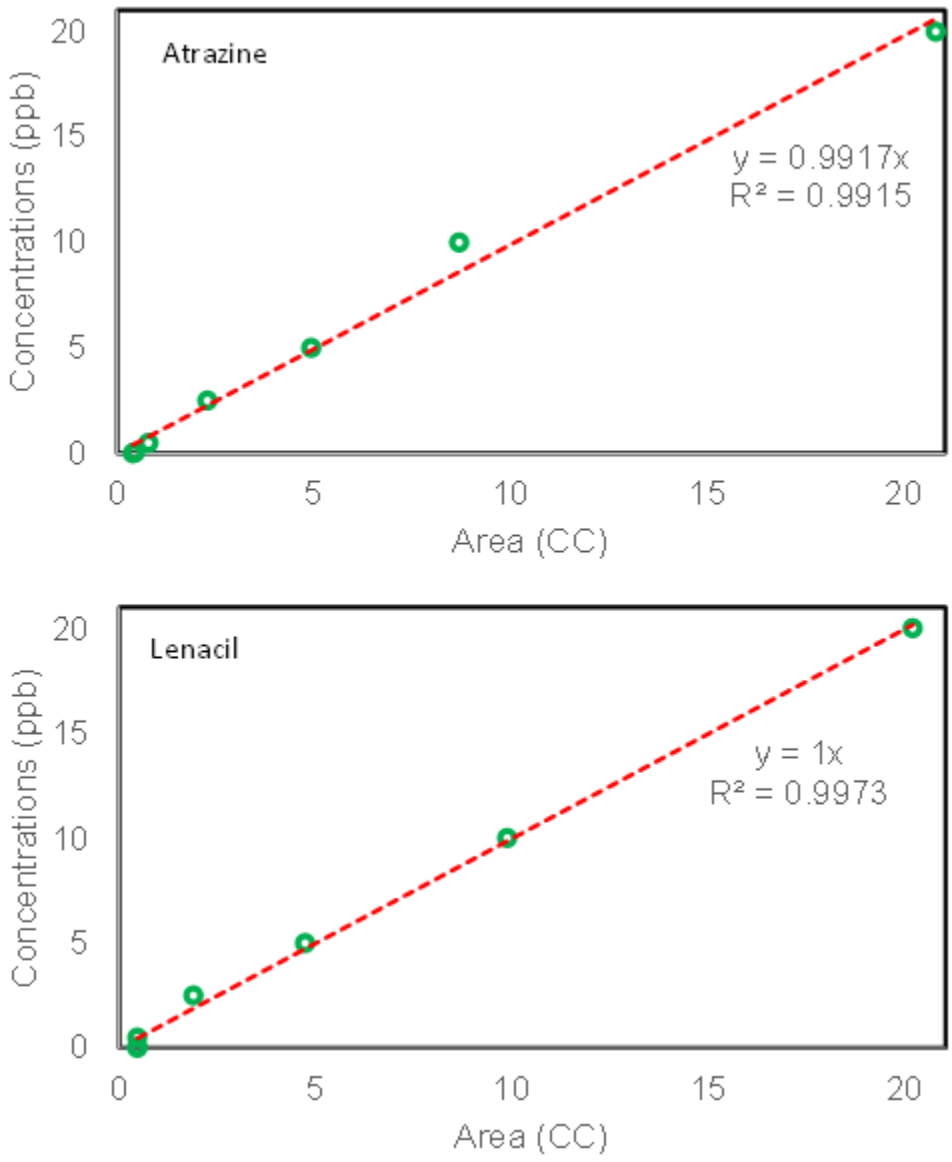


Figure 6. The calibration curves of Atrazine and Lenacil. Top linear curve shows the linear relationships between concentrations of Atrazine and corresponding GC-MS-MS peak area of Atrazine. Bottom linear curve shows the linear relationships between concentrations of Lenacil

and corresponding GC-MS-MS peak area of Lenacil. The linear fit with R2 values of greater than 0.99 shows the strong relationships and successful method.

4. CONCLUSIONS

From years of research and development of herbicide analysis in water sample, the method proposed here has several advantages over other techniques such as ELISA and LC-MS-MS methods.

- The solid phase extraction (SPE) of water sample is simple and it can handle dirty environmental water samples.
- This SPE process can handle a large volume of water (3 mL-200 mL).
- It uses low amount of solvent and cartridge are robust and results are reproducible.
- The same SPE method can be used for multiple types of pesticide residue analysis.
- The GC-MS method used for the analysis requires 20 minutes to complete the analysis of one sample, which is relatively a shorter method.
- Multiple herbicides and residues elute within 20 minutes in this GC-MS method.
- This GC-MS method can be used for analyzing multiple pesticide and herbicides simultaneously.

We anticipate that the simple SPE and GC-MS methods of this research will be useful for the detection of these herbicides/pesticides and fungicides residues in water samples of various types of environmental water bodies.

ACKNOWLEDGEMENT: Authors acknowledge the funding support from Division of Boating and Waterways, California Department of Food and Agriculture, California state government, which facilitated the method development.

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