Methods development and application for triazines determination in fruits and vegetables: Comparing the significance of solid phase extraction, QuEChERS extraction, and ultrasonic solvent extraction techniques

By

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Methods development and application for triazine herbicides determination in fruits and vegetables: Comparing the significance of solid phase extraction, QuEChERS extraction, and ultrasonic solvent extraction techniques

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Declaration

I, Hopewell Mlungisi Mnyandu, declare that this research project is entirely my original work, excluding where stated otherwise and the citations were used to acknowledge other people's work used in this dissertation. The dissertation has not been submitted elsewhere for any other degree/diploma here or other universities for examination.

(Signature of candidate)

(Supervisor)

…….22-May-2021…… (Date)

Dedication

To my late mother (Nomusa D. Mnyandu) and late father (Zimisele A. Ninela), and the rest of the Mnyandu, Gule, and Biyela families.

Be Humble in Victory. Give God All the Glory.

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Abstract

This work reports on a quick, easy, cheap, effective, rugged, and safe (QuEChERS), solid phase extraction (SPE) and ultrasonic solvent extraction (USE), combined with liquid chromatography-photodiode array detection (LC-PDA) method optimization for the analysis of triazines pesticides. These LC-PDA analytical methods were optimized in terms of linearity while SPE, USE, QuEChERS were optimized in terms of limits of detection (LOD), limit of quantification (LOQ), percentage recoveries in order to improve their efficiency. The optimized methods were then applied to fruits and vegetables from local markets to assess the residues of the commonly used triazine pesicides (atrazine, simazine, propazine, ametrine, terbuthylazine).

The results showed a good linearity with the R^2 values above 0.99 for all the triazines analysed. The LODs and LOQs ranged from 0.4 -1.4 µg/kg and 1.5 - 4.5 µg/kg for QuEChERS, 0.3 - 1.8 µg/kg and 1.4 - 4.9 µg/kg, for SPE. The recoveries ranged from 84 -102% for QuEChERS and 76 -119% for SPE, with relative standard deviation less than 20% for results of both methods. These results showed that both QuEChERS and SPE proposed methods are sensitive indicating that they can be effectively applied for the detection and monitoring of the selected triazines in fruits and vegetables. The optimised QuEChERS and SPE methods were then applied to fruits (apples, pears, bananas, avocado, and oranges) and vegetables (carrots, potatoes, tomatoes, cucumber and spinach) to compare their applicability. The concentration of triazines were detected in spinach (17 - 84 µg/kg), avocado (4 - 6 µg/kg), cucumber (10 - 14 µg/kg), tomato (34 - 39 µg/kg), carrot (22 - 71 µg/kg), banana (19 – 38 µg/kg), orange (23 – 46 µg/kg), apple (10 - 16 µg/kg) and pear (18-20 µg/kg). Simazine was detected in all fruits and vegetable samples except in pear, while terbutylazine was not detected in all samples analysed. Propazine and ametryn were only found in carrot while pear sample only had atrazine. The mean results of both methods were not statistically different at 95% confidence level. QuEChERS can be recommended for routine analysis of these triazines since it involves fewer extraction steps compared to SPE and thus will require shorter analysis time.

The ultrasonic solvent extraction (USE) method was also optimized and applied with and without solid-phase clean-up for analysis of triazines. The LOD and LOQ obtained ranged from 1.1-1.8 µg/kg and 3.4 - 5.2 µg/kg for USE with SPE clean-up and 0.6 - 1.0 µg/kg and 1.7 - 2.9

µg/kg for USE without SPE clean-up. The methods showed recoveries ranging from 75 - 81% for USE with SPE clean-up, and 102 - 106% for USE without SPE clean-up with relative standard deviation less than 15% for both methods. This implied that both methods are precise, however, USE without SPE clean up showed to be more sensitive for the extraction of the selected triazines pesticides in fruits and vegetables. The USE with and without SPE clean-up methods was then applied to fruits (grapes, lemon, passionfruit and plum), and vegetables (beetroot, bell pepper, cabbage and peas) samples. The concentration of triazines were detected in beetroots (102 - 152 µg/kg), bell pepper (45 - 88 µg/kg), grape (14 - 22 µg/kg), lemon (3.2 -156 µg/kg), passionfruit (32 - 222 µg/kg), and peas (126 – 138 µg/kg) and plum (9 – 11 µg/kg). Propazine was the most dominant triazine while terbuthylazine was not detected in all samples analysed. Passionfruit was the most polluted while none of the analysed triazines was detected in cabbage. All the triazines were quantified at levels below the maximum residue limits (MRL) in all fruits and vegetables which reveals that they are not harmful to human health. This work showed that proposed USE method can be considered an inexpensive, environmentally friendly method that can be used for routine analysis and monitoring of the selected triazines. Moreover, USE can be accurately applied without the additional SPE to assess the residual levels of triazines in fruits and vegetables.

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Abbreviations

ACN	Acetonitrile
AOAC	Association of Official Analytical Chemists
APCI	Atmospheric Pressure Chemical Ionization
ECD	Electron Capture Detector
EPA	Environmental Protection Agency
ESI	Electrospray Ionization
EU	European Union
GC	Gas Chromatography
HLB	Hydrophilic-Lipophilic Balance
HPLC	Higher Performance Liquid Chromatography
LC	Liquid Chromatography
LOD	Limits of Detection
LOQ	Limits of Quantification
MRL	Maximum Residue Limit
MS	Mass Spectrometry
PDA	Photodiode Arrays
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
RSD	Relative Standard Deviation
SPE	Solid Phase Extraction
USA	United States of America
USE	Ultrasonic Solvent Extraction
UV	Ultraviolet

UV-Vis	Ultraviolet-Visible
WHO	World Health Organization
WWTPs	Wastewater treatment plants

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Chapter One: Introduction

1.1 Background

South Africa is a diverse country that is home to more than 60 million people and has a diverse environment with climate conditions favoring the farming of different types of plants including maize, trees, tropical fruit, sugar cane, etc. (Quinn et al., 2011). However, the increase in human population has resulted in high demand for food especially crops, thus pesticides are often used to maintain both agricultural productivity and human health (Popp et al., 2013). Pesticides have been recognized as an important measure to meet the growing concerns of food commodity shortages as the growth of the global population increases. Moreover, in sub-Saharan Africa, South Africa is one of the countries that import huge amounts of pesticides, with over 500 registered pesticides (Quinn et al., 2011). Pesticides help farmers or producers by preventing or reducing the losses caused by pests to agricultural produced crops, therefore resulting to higher yield and the availability of food at affordable prices all year round (Cooper and Dobson, 2007).

Pesticides are chemical substances applied to crops to eliminate agricultural pests and weeds that compete with food crops, and spread diseases, thus improving plant quality and shelf life (Bakırcı et al., 2014). There are various classes of pesticides that function differently in terms of the targeted pests. These include fungicides (fungi), insecticides (insects), herbicides (plants or weeds) nematicides (nematodes or worms), rodenticides (rodents), avicides (birds), algicides (algae), bactericides (bacteria), molluscicides (snails or slug), acaricides (spiders), and miticides (mites), (Bateman et al., 2016). Triazines are pesticides that are classified as herbicides and are known as persistent organic chemical compounds; thus, they are more susceptible to either biological or chemical degradation (Klementova and Keltnerova, 2015). They are used in agricultural or non-agricultural areas to control unwanted plants such as broadleaf weeds and annual grasses (Mtyopo, 2004). Triazines tend to inhibit photosynthesis (specifically the photosynthetic electron transport) and therefore, eradicate weeds. Even though triazines development was meant to poison the unwanted plants selectively, they also have effects to the consumers (humans and animals), (Mtyopo, 2004; Beceiro-González et al., 2014). Triazines adverse effects could be immediate (acute) or long term (chronic) effects including skin problems, eye irritation, headache, nausea, cancer, birth defect, disruption endocrine system, and interruption of hormone functions, (Bakırcı et al., 2014; Fang et al., 2014).

Though triazine usage is important for crops protection, their overuse or misuse by farmers has led to their accumulation in produced crops which including fruits and vegetables, and thus have been detected in processed food products including fruit juices. This is because triazines can adsorb to the surface/skin of the fruit or vegetable and eventually get absorbed through stomata, the cuticle of the leaves, and can be absorbed by root hairs and get translocated by the phloem to the produce (Ando, 2019). It is therefore important that the triazine residues that remain on or in food crops after application be monitored whether their concentrations do not surpass their admissible levels known as maximum residue limits (MRLs).

The monitoring of the triazines is conducted by first extracting the compounds of interest from the matrix and then analyzing them to obtain the concentration at which they are present. Due to the matrix effect in fruits and vegetables, the extraction method must be very selective and sensitive for successful analysis (Tankiewicz, 2019). Also, the triazines are usually present in low concentration levels which makes it more difficult to determine them with the present methods. Therefore, alternate new or improved extraction and detection analytical methods that are simpler, faster, and improving recoveries for difficult matrices are crucially needed (Lesueur et al., 2008). Analytical methods such as liquid chromatography (LC) with MS or ultraviolet (UV) including diode array detectors (DAD) and a gas chromatography with mass spectrometry (GC-MS) are being considered for separation and detection of pesticides because they are more selective and sensitive than other tradition techniques (Moliner-Martínez et al., 2015).

This work therefore focused on qualitative and quantitative analysis of triazines in fruit and vegetables from local markets. The triazines (simazine, atrazine, ametryn, propazine and terbuthylazine) were extracted using QuEChERS (quick, easy, cheap, effective, rugged and safe) method, solid phase extraction (SPE), ultrasonic solvent extraction (USE) followed by liquid chromatography (LC) coupled to photodiode array (PDA) for the analysis. The selected extraction techniques were chosen based on their ability to purify extract from food which leads to lower detection limits and higher recoveries of the analytes (Pawliszy, 2012). The LC was preferred compared to GC for the selected triazines due to their low volatility. Even though MS is most sensitive, its applicability is often limited compared to PDA due to its high costs and thus it is not available in many laboratories. Hence, PDA was used in this work with the aim of developing affordable method. The triazines of interested were chosen due to their wide usage in crops as pre and post emergent pesticides.

1.2 Problem statement

As human population continue to rapidly increase, the demand for food especially crops has increased which has led to the application of more pesticides to increase crop protection and yield. However, over usage of pesticides in fruits and vegetable farming can result in their residues remaining on or in them beyond harvest time. The residues that remain are associated with adverse health impacts to the consumers (humans and animals) if the residues are above the MRLs. Therefore, pesticides analysis in fruits and vegetables from our markets is of paramount importance to determine if their concentrations are within the acceptable levels that are safe for human consumption. Since pesticides are present at very low concentrations and the fruits/vegetable samples are complex, their analysis requires intensive analysis method development for their extraction, separation and detection. This results in obtaining appropriate sample preparation procedures with improved detection limits and extraction efficiency for effective extraction of the analytes from the sample matrix. The determination is successfully completed by analysing the analytes in the extract using a sensitive separation and detection method which will results in accurate quantification of the analytes residues at very low concentrations.

Therefore, this research focused on method development for QuEChERS method, solid phase extraction, ultrasonic solvent extraction and liquid chromatography coupled with photodiode array detector for determination of triazines residues in fruits and vegetables. The extraction techniques were first optimized in order to improve their extraction efficiencies and detection limits, while LC-PDA was optimized to improve its separation efficiency and accuracy. These methods were then applied to real samples from local markets to assess the presence of the triazines of interest and to compare if their concentrations are within the MRLs values to evaluate their consumption safety. To best of our knowledge no work has been reported in literature on the levels of triazines in fruits and vegetables from the selected local markets, moreover, not much has been conducted in KwaZulu Natal Province which is the study area.

1.3 Aim and objectives

1.3.1 Aim

To optimize and apply the QuEChERS, SPE, and USE methods coupled to the LC-PDA method for the effective determination of triazine herbicides in fruits and vegetables.

1.3.2 Specific objectives

1. To optimize the LC-PDA technique for proper separation and accurate identification and quantification of the target triazines.
2. To optimize the QuEChERS, SPE, and USE method using spiked fruit/vegetable samples to increase method's efficiency for all triazine compounds of interest.
3. To apply the optimized methods for the quantification of triazine pesticides from real food matrices (fruits & vegetables) purchased from the local supermarkets in Pietermaritzburg (KZN), South Africa.

1.4 Hypothesis

A significant number of fruits and vegetables from our local markets (Pietermaritzburg (KZN), South Africa) are possibly contaminated by triazine pesticides above the MRLs.

1.5 Research questions

- Which QuEChERS, SPE, and USE conditions need to be optimized for the accurate quantification of triazine pesticides from fruits and vegetables?
- Are the selected triazine compounds present in fruits and vegetables purchased from the local supermarkets?
- What are the concentrations of these selected triazines compounds in fruit and vegetable samples and are they within the allowable maximum residue limits?
- Which extraction technique is more efficient between QuEChERS and SPE for the extraction of the target triazines?
- Will USE method be more efficient with or without SPE clean-up for the extraction of the target triazines?

1.6 Research justification

Agriculture is the major source for food supply in most nations, especially in developing countries. Therefore, there is a demand for improving the agricultural sector to cater for the increasing world population that is currently 7.7 billion and expected to reach 9.2 billion by the year 2050, (Francis et al., 2020). The increase of crop production yields using pesticides, fertilizers has been established in developing countries to meet the food demands. However, relying on chemical pesticides has been found to prompt a major health hazard towards human wellbeing as well as environmental issues (Francis et al., 2020). This is due to fact that pesticides residues remain on the produce and thus be consumed by humans where they may

result in carcinogenic, respiratory, endocrine, reproductive dermatological, gastrointestinal, and neurological effects (Nicolopoulou-Stamati et al., 2016).

Therefore, the importance of this research to monitor the triazine pesticides in fruits and vegetables from our local markets to assess if their concentrations are acceptable for human consumption. Hence, this project provides knowledge on the pesticide levels in fruits and vegetables to alert if there is any health risk to the consumers. Also, the results from this work have been published and has been added to the pesticides data base which can assist policy makers to set allowable limits that are specific for African countries. QuEChERS being a quick, cheap, easy, effective, rugged and safe method (commonly used for the extraction of triazines in food matrix) was used and compared with the SPE being a selective sample preparation and purification method (commonly used for extraction of water matrix) in order to evaluate which of the two methods is more efficient for the extraction of the target triazines in the selected fruits and vegetables. The USE (being the cheaper and available technique) was evaluated with and without SPE clean up in order to assess if its extraction efficiency is improved by the clean-up step.

1.7 Research summary

This research consists of six chapters. **Chapter one** covers the background and problem associated with triazine pesticides. The aim and objectives resulted from the problem statement. As well as the research questions that were answered by this work and the research justification ware covered in chapter one. **Chapter two** covers the literature review; more background on pesticides, uses, their impacts, and the fate of the pesticides on the environment. Sources, exposure pathways, physical parameters, and maximum residue limits were covered also. A review of techniques from sample preparation, separation, and detection that were utilized in the world for the extraction of pesticides in different matrices was covered in chapter two. **Chapter three** consists of methods and materials used in this work; experimental procedures, Instrumentation used**,** chemicals/reagents, standards preparation, sampling, sample preparations, and clean-up. Method validation and application to real samples were also covered in chapter three. **Chapter four** reports the results and discussions in a journal article format. Where techniques such as QuEChERS and SPE methods followed by LC-PDA were optimized and applied to fruits and vegetables samples obtained from Pietermaritzburg local supermarkets. **Chapter five** reports the results and discussions in a journal article format. Where methods such as ultrasonic solvent extraction with and without solid-phase clean-up

were used to determine triazines pesticides in fruits and vegetables samples. **Chapter six** consists of the conclusion and future recommendations.

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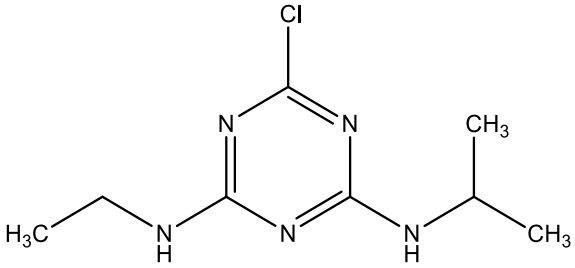
Chapter Two: Literature Review

2.1 Background

Pesticides are substances used in agriculture to eliminate or control the development of unwanted plants, pest manifestation, and vectors of disease that compete with humans for food, destroy property, and spread/carry diseases. These include fungus, microbes, insects, plant pathogens, weeds, plant diseases, snails, slugs, mollusks, birds, mammals, fish, and nematodes, etc. (Yadav and Devi, 2017). There are different types of pesticides which are named according to their function of targets, these include insecticides, fungicides, herbicides, bactericides, miticides, rodenticides, avicides, molluscicides, algicides, acaricides, and nematicides (Bateman, 2016). Triazine pesticides belong to a herbicides family and are used to control weed. They can be classified as soil- or foliage-applied compounds that are usually absorbed by roots or leaf tissues respectively (Tian et al., 2014; Haque et al., 2018). They provide selective weed control in agricultural plants which includes fruits, corn, vegetables, sorghum, and sugarcane. Selective herbicides are preferred over nonselective or total herbicides since they can control weeds without affecting the crop, while nonselective or total herbicides can kill all vegetation. Their selectiveness depends on factors such as their uptake by plant, translocation, or metabolism, and the differences at the site of action. Triazines are chemically stable and very persistent in the environment. They have moderate to high water solubility and slightly binds to soil particles and therefore can leach from soil into the water bodies such as surface water and groundwater. When triazines and their degradation products are ingested, they have been shown to have acute and chronic negative impacts on the health of humans, animals, and aquatic life (Tian et al., 2014; Haque et al., 2018).

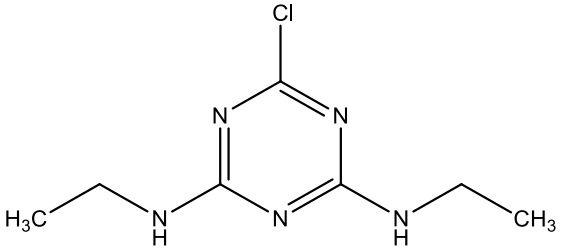
2.2 Uses of pesticides

Triazine herbicides are either pre-or post-emergence herbicides that are used to kill weeds before they emerge from the soil or can eradicate weeds after they have germinated or emerged from the soil (Breckenridge, 2010). The triazine herbicides analyzed in this work can be categorized as chloro-s-triazines (atrazine simazine, terbuthylazine and propazine) and thiomethyl-s-triazines (ametryn), (Figure 2.1).



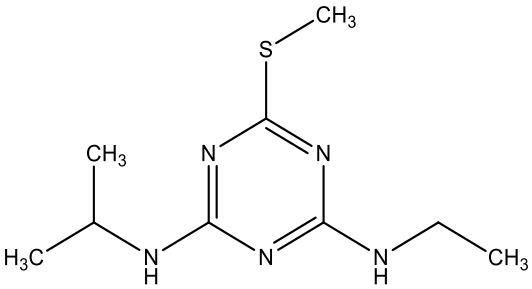
Atrazine

IUPAC name: 6-chloro- 4-N-ethyl-2-N-propan-2-yl-1, 3, 5-triazine-2, 4-diamine.



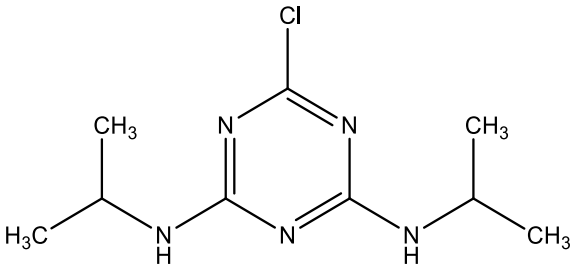
Simazine

IUPAC name: 6-chloro-2-N,4-N-diethyl-1,3,5-triazine-2,4-diamine



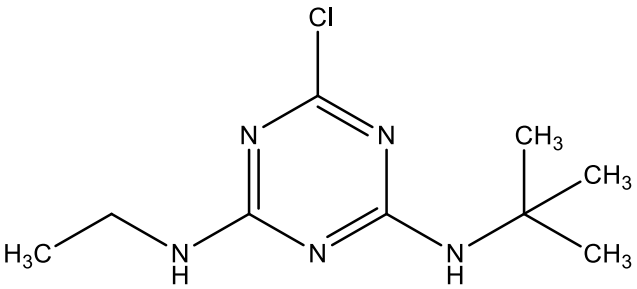
Ametryn

IUPAC name: 4-8 -N-ethyl-6-methylsulfanyl-2-N-propan-2-yl-1, 3, 5-triazine-2, 4-diamine



Propazine

IUPAC name: 6- chloro-2-N,4-N-di (propan-2-yl)-1,3,5-triazine-2,4-diamine



Terbuthylazine

IUPAC name: 2-N-tert-butyl-6-chloro-4-N-ethyl-1, 3, 5-triazine- 2, 4-diamine

Figure 2.1: Structures of triazine herbicides (Rodríguez et al., 2013)

Simazine is a selective systemic triazine used before weed emergence and is known to prevent photosynthetic activity by producing free radicals (Zaady et al., 2004). Simazine is mainly applied in agricultural or non-agricultural fields to control unwanted plants and grass. The

crops that are vastly sprayed with simazine include corn, sorghum, sugar cane, artichokes, asparagus, berry fruit cherry, grape, almond, apple, avocado, hazelnut, peach, walnut citrus crops, coffee, hops, oil palms, olives, vegetables and ornamental crops, turfgrass, orchards, vineyards and forage crops (World Health Organization, 2003; Heri et al., 2008). Simazine herbicide is also used as a nonselective weed controller in industrial areas and can be used to control submerged weeds as well as algae in places such as aquariums, farm ponds, swimming pools, and cooling towers, etc. (Pohanish, 2015). In California, (USA) simazine was one of the most used pesticides in the year 2003, primarily in fruit and vegetable crops (Gunasekara et al., 2007).

Ametryn is a very selective pesticide that is applied to crops such as corn, popcorn, banana, pineapple, to eliminate weeds with large leaves and grasses before and after emerging (Simoneaux and Gould, 2008). It is mostly used in sugarcane as a pre-emergence treatment because it offers good and short-term residual activity and has good foliar activity on broadleaf weeds and grasses (Smith et al., 2008). It is also used in citrus, palm, and coffee and slightly used as a post-directed spray in corn. (Heri et al., 2008).

Atrazine is an herbicide that is used to inhibit photosynthesis of the unwanted plants and ultimately eradicate them (Pathak and Dikshit, 2011). Atrazine is applied before and after weed germination to inhibit weeds with large leaves from growing in agricultural fields and roadways (Hodgson, 2012). It is also used in corn, sugarcane, sorghum crops, forests, and other non-agricultural areas such as golf courses, rangeland, near high-voltage power lines, and other recreational areas, etc. (Rinsky et al., 2012).

Propazine is a selective herbicide commonly used to inhibit photosynthesis of the target plants after being absorbed by their leaves and the roots (Maples, 2014). Propazine is commonly used to manage weeds with large leaves and to remove grasses with a lifecycle not lasting more than a year before they emerge in areas where vegetation eradication is desired. It is also used in greenhouses or desired areas either before, during, or after plantation (crop emergence) (Maples, 2014).

Tertbuthylazine is a selective herbicide used as a post-emergence with excellent broad-spectrum weed control in different types of crops (Heri et al., 2008). It is used in crops that

include peas, sorghum, sugarcane, lupin, olives, citrus, pineapples, corn, bean, potatoes, pome fruit, and grape in vineyards and orchards (Grenni et al., 2012; Watt et al., 2010).

2.3 Effect of pesticides

The most common effect of triazine herbicides is known to be photosynthesis-inhibiting which blocks the photosynthetic process so that captured light cannot be used to produce sugars which is a process that is vital to plant survival (Draber et al., 1991).

Simazine short-term exposure on rats over 500 mg/kg dose showed that is lethal for 50% of the rat's population (World Health Organization, 2003). Carcinogenic impact of simazine treated rats was investigated and female rats showed an increase in mammary tumors as the doses of simazine increased. In males treated rats, there was an increase of adenomas and carcinomas of the liver, and in pancreatic tumors and tumor of adrenal gland tissue, as the doses of simazine increases. No deaths nor toxic effects on dogs that were exposed to lower doses of simazine over the long-term but exposure to higher doses resulted in cachexia (fatigue and weight loss), and the reduction of erythrocyte (red blood cells) (World Health Organization, 2003). In mice, simazine induced a mutagenic effect which was caused by the rupturing, repairing, or the exchange involving DNA molecules in the uniform area of the chromatids that increase drastically. The induced chromosome number deviation was observed in plants exposed to simazine. It was found that about 124 workers manufacturing simazine had dermatitis which turned severe forming skin redness, oedema (fluid retention), rash, and eventually blisters after 7-10 days of exposure (World Health Organization, 2003).

Ametryn is class III in toxicity which means they are non-toxic to mammals and fish but toxic to mollusks (i.e. clams, mussels, oysters, and scallops, as well as octopus, snail, and squid, etc.) and crustaceans (i.e. crab, crayfish, lobster, prawns, and shrimp). In fish species such as goldfish, bluegill exposure at a higher dose can lead to death in a space of 4 days of exposure (Farré et al., 2002).

Atrazine is moderately toxic, however, exposure to its high concentrations (≥3 ppb) can cause carcinogenic effects in humans (Pathak and Dikshit, 2011). Atrazine is associated with adverse health effects such as tumors, reproductive tumors, breast, uterus, and ovarian cancers and can also cause leukemia and lymphoma. Atrazine can alter hormonal functions, result in humans and amphibians weight loss, and can cause birth defects and the disruption of the endocrine

system (Hodgson, 2012). In a study conducted by Whalen et al., (2003), dogs and rats showed food intake reduction and body weight loss when exposed to high doses of atrazine chronically. The rats that were orally exposed to atrazine in large doses showed breathing difficulties, convulsions, hypoactivity, muscle weakening, and death. United States Environmental Protection Agency (US-EPA) has declared that atrazine is carcinogenic in humans and other mammals at high doses, but the mutagenic and teratogenic effect was not evident (IRED, 2006). After a short time of exposure to atrazine, rat strain had an incidence of mammary and pituitary tumors in the brain. Atrazine exposure to female rats during lactation tends to suppress the sulking-induced prolactin release. While male offspring were exposed to atrazine through lactational exposure from females/ mothers that were exposed to atrazine had an increase in prostatitis incidences (Sanderson et al., 2001).

Propazine is associated with neuroendocrine mechanisms of toxicity and was found to have a disruptive effect on the estrous cycle and can impact the reproductive cycle in females, while in males can impact sexual development (Maples, 2014). Propazine can also impact the pituitary glandular system. Propazine is also known to alter certain B vitamins, such as thiamine and riboflavin, and to block the metabolism of sugar and carbohydrate. Studies showed rodents to have tumorigenic effects when exposed to propazine, therefore, there is a carcinogenic potential in humans. Propazine was also found to be toxic to aquatic animals after exposure to a concentration of 3 mg/L which resulted to the death of 50% of the test fishes within 7 days. In addition, propazine blocks sugar and carbohydrate metabolisms and may also alter the metabolism of certain B vitamins, including thiamine and riboflavin (Maples, 2014). Workers manufacturing propazine reported that propazine may also cause skin and eye irritation within a short term of exposure. While long term exposure targets skin and reproductive cells and may cause the development of tumors and skin allergy, reproductive effects, and fetal effects (Pohanish, 2015).

Terbuthylazine is classified as a chloro-s-triazine herbicides that are known to prevent photosynthesis at photosystem II (a first protein complex in the reactions of oxygenic photosynthesis that uses light as a catalyst) in weeds (Želježić et al., 2018). It is a selective herbicide for vegetation management and is classified as highly hazardous (Watt et al., 2010). Terbuthylazine is suspected to cause lung cancer and non-Hodgkin's lymphoma which is white blood cancer (Mladinic et al. 2012). Terbuthylazine and its metabolites or degrades were found to alter the development (fetus), growth, and survival of aquatic species in a study focusing on

the toxicological effects of terbuthylazine. This led to pathological alterations in the tissues and organs and causing oxidative stress since terbuthylazine induced changes in biochemical and hematologic parameters (Želježić et al., 2018).

2.4 Fate of triazines in the environment

Simazine is much persistent in the environment since it does not absorb into soil particles or sediments and is most likely to contaminate the groundwater. The groundwater contamination by simazine was reported in European countries such Germany and Italy, and about 1-2 µg/L of simazine was detected in groundwater in the USA (World Health Organization, 2003). Simazine has low solubility in water but is considered a leacher and it can stay in the soil for months since its half-life in soils is 1.5-6 months. Simazine's volatilization and photodegradation are not prominent under normal weather conditions but can degrade through hydrolysis (World Health Organization, 2003).

Ametryn can persistent in the environment and can be degraded in the soil by microbial degradation (Farré et al 2002). Ametryn has high water solubility and can move in the soil vertically or literally. It is presence in the fresh waters and the marine coastal environments is due to its ability to leach by high rainfall, channel irrigation, and floods. The presence of ametryn at lower residue levels in the aquatic system is because of diffuse pollution, leaching, and atmospheric deposition and can move from wastewater treatment plants (WWTP). This is due to that ametryn escapes or survive degradation from WWTP due to higher residue levels that are present in the effluents (Farré et al 2002). A study published by Farré et al (2002), found that the biodegradation of ametryn in the activated sludge is slow since it took 12 days to reach 60% degradation, and thus ametryn can persist longer in the aquatic environment. With the island climatic conditions favoring the rainfall, most of the pesticides are transported to the rivers through runoff and are also absorbed by the soils causing contamination of aquatic, and marine environments of the island respectively (Bocquené and Franco, 2005).

Atrazine has a slow rate of dissipation from the environment, therefore, can persist. This is because it is resistant to abiotic hydrolysis and direct aqueous photolysis but moderately prone to aerobic biodegradation (Liu, 2014). Atrazine is moderately soluble in water and soil particles cannot absorb it. Atrazine can be transported in a dissolved form and is likely to leach or move with runoff and hence be detected in many water bodies such as groundwater and surface water.

Atrazine can persist in the soil from 1 week up to two months. Due to atrazine resistance to degradations it has a 3 – 11 months half-life, and atrazine is found to degrade slowly in water bodies (1 – 2 years) (Pathak and Dikshit, 2011).

Propazine is directly applied to soil and weakly binds to the soil particles and has low water solubility thus can persist and move through the soil moderately. Propazine can leach into groundwater in areas where there are sandy soils, high rainfalls, and irrigation. This because propazine resists degradation by hydrolysis, photolysis, and biodegradation thus persist longer especially where climatic conditions are dry and cold. Propazine can dissipate in the environment by soil microbes at a moderate rate under aerobic conditions in the soil. Its degradations in sterile loamy sand and nonsterile loamy sand have a half-life of 2-3 months and 3-6 months.

Terbuthylazine can be degraded or metabolized by soil microorganisms and has a half-life of 1 – 2 months in biologically active soils thus can persist in the environment (Watt et al., 2010). Terbuthylazine moves less within the soil profile than atrazine and the highest soil residues are usually found in the topsoil layer (James et al., 1998). According to Grenni and co-workers (2012), terbuthylazine fate and behaviour in soil have raised environmental concern and it is evident that over 0.1 µg/L (an MRL value established by the European Union agency (EU) for each pesticide in drinking water) levels of terbuthylazine have been found in surface and groundwater.

2.5 Exposure pathways of pesticides

Humans and animals can contact triazine pesticides directly or indirectly. When applied triazines drift away (via wind) from the intended area. This may lead to direct contact with the human through breathing fume or skin contact. Also, applied triazines may be forced by runoff into local water bodies, or the groundwater, resulting in secondary poisoning when the contaminated waters are consumed. In some cases, animals could be exposed through the spraying of pesticides which eventually lands to them directly, or indirectly consuming contaminated plants or prey that was exposed to pesticides. Pesticide can enter a human body or animal through oral, respiratory, or dermal exposure (Schulze et al., 1997). An **oral entry** occurs through the mouth especially by drinking water or eating contaminated food. Also, when a person has pesticides in the hands, and they lick or place a cigarette in the mouth that is contaminated by pesticides. **Respiratory entry** is where pesticides sprays, powder, or

vapours enter the system through breathing by either mouth or nose. **Dermal entry** is associated with eyes and skin contact with pesticides substances in a form of sprays that lands on the body and they mostly absorb quickly through forearms, eyes, and forehead. These exposures can accumulate through careless use of pesticides, for instance storing or placing pesticide in bottles (used for drinking) as well as not using appropriate clothes (protective gear) or equipment such as gloves and masks during the application of pesticides (mixing or spraying). Spraying in windy conditions causes the spray to drift away to other areas and can increase the chance of someone being harmed by accidentally absorbing orally, dermal, or respiratory some of these pesticides (Schulze et al., 1997). Oral, dermal, and respiratory pesticide exposure are represented in Figure 2.2.

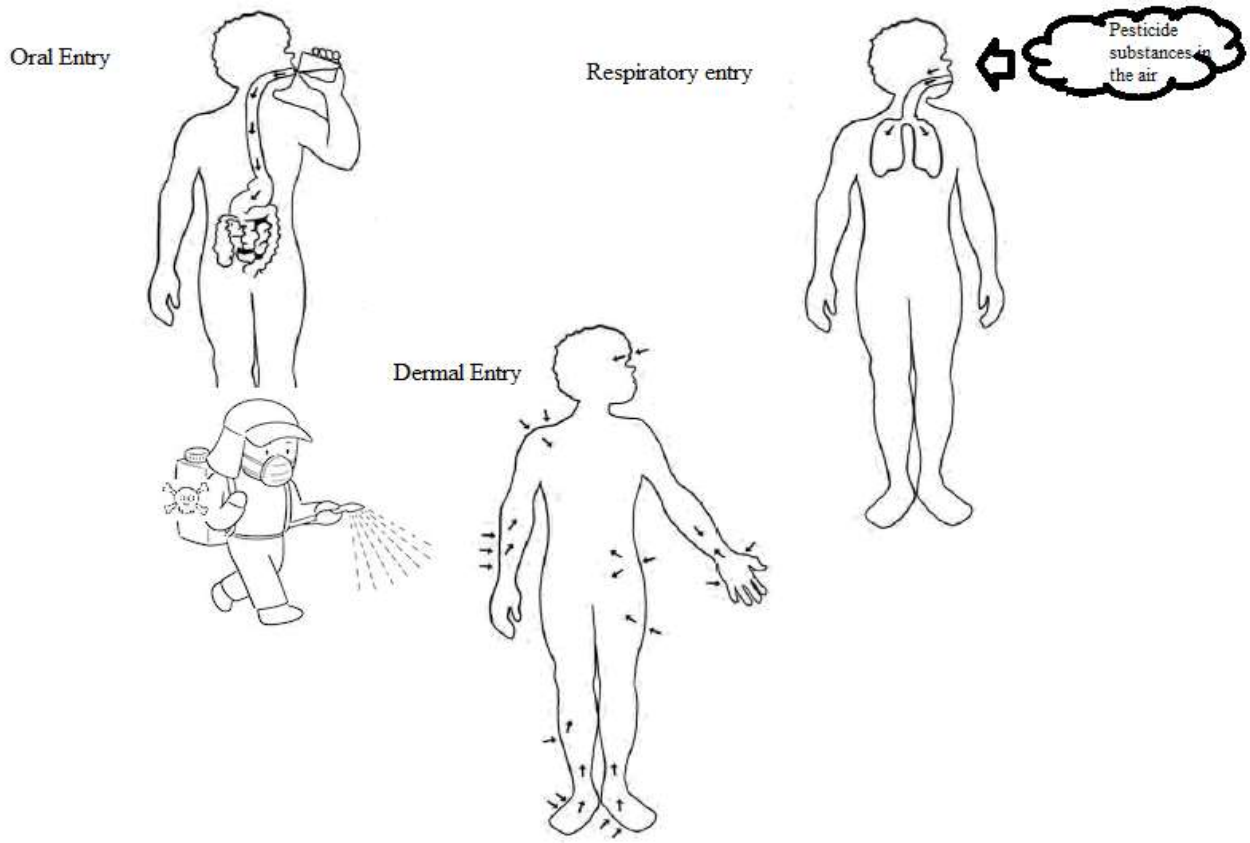


Figure 2.2: Exposure pathways of pesticides <www.health.gov.au>

A study by Tariq et al., (2007) revealed that the overuse or misusing pesticides which include high sprays volumes than the accepted concentrations in cotton plants showed high risks to field workers and pickers and risks from unaccepted concentration residue in cottonseed oil and cakes produced in a farm around Punjab and Sindh (Pakistan). This finding showed that overuse or misuse of pesticides farmers have high work-related exposure potential which could

lead to short-term (acute) and long-term (chronic) effects (Tariq et al., 2007). The study conducted in the Washington States by Simcox et al., (1995) revealed that children are highly contacting to high doses of pesticides from household dust and soil in agricultural homes. This indicates that children of agricultural families are exposed to high levels of agricultural compounds even though they do not participate in farming. Boobis et al (2008) detected pesticides residues in food items containing more than one active pesticide in higher concentrations than the MRLs of the European Union which is against the US Food Quality Protection Act (FQPA) of 1996. This implies that there is also an increased risk of exposure to multiple pesticide residues in the diet due to the dose addition of these compounds (Boobis et al., 2008).

2.6 Sources of pesticides

Pesticide that are found in the environment are due to human activities. These activities include the application of pesticides in agricultural crops, soil erosion due to deforestation, and domestic or industrial discharges. The movement of pesticides on our daily basis can be tracked in a form of a cycle, for example, the soil which acts as a filter, buffer, and degrades to stored pollutants with the help of organic carbons present in the soil (Fenik et al., 2011). Soil is the main source of pesticide contaminating air, water, human and plants by leaching them to water bodies or through, subsurface drainage, interflow, and runoff. Mineral nutrients and pesticides from the soils can also be transferred directly into plants and indirectly to animals consuming contaminated plants, which both eventually get consumed by humans. The slow degradation of pesticides in the soil is the leading cause of such contaminations. Therefore, contaminated soil can transport pesticides to the plant through absorption by roots of the plant and move up through the stem to leaves and fruits. So, when an animal or human feed from the plant can be at risk of being orally exposed. Pesticides can be deposited into the water through runoff or leaching or in a form of sewage produced by humans after the consumption of pesticides. The contaminated water is then evaporated to pollute the air, which can redeposit pesticide in a form of rain back to the soil and water sources. The pesticides movement can cycle in the environment following that manner or continuously (Fenik et al., 2011). Even people who prefer to eat organic food (pesticide-free food) are also exposed to pesticides in a form of drinking tap water, which is purified from sewage water (Fenik et al., 2011). The process of the movement of pesticides from the environment then to people or vice versa is represented by the circle shown in Figure 2.3.

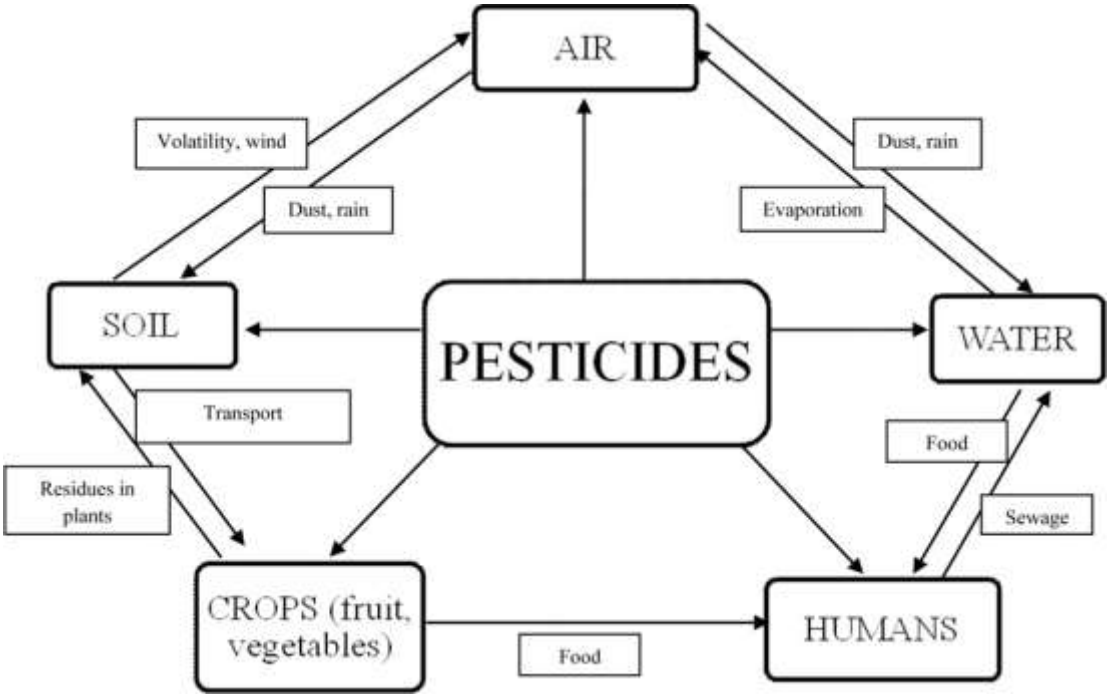


Figure 2.3: Cycle representing the movement of pesticides in our daily basis (Fenik et al., 2011)

2.7 Pesticides in fruits and vegetables

Pesticides in the soil can be absorbed through the root of the plants or foliage, then translocate upward and accumulates in the newly formed leave and the growing tips or shoots (branches and fruits) of the plants (Trapp and Legind, 2011). Pesticides in the environment can also enter the plant through uptake from passive transport. These processes include transpiration that initiate the absorption of water, diffusion of contaminants from the soil to the roots or they can attach to soil particles and eventually diffuse into the plant tissue. Pesticides pollutant present in the air (in a form of dust or vaporized substances) can be absorbed by a plant through diffusive gas exchange with air or via wet and dry particle deposition from the air onto the plant surface which is then followed by diffusion of contaminants into the plant tissue (Trapp and Legind, 2011). The transport and uptake in the soil-air-plant system processes are shown in Figure 2.4.

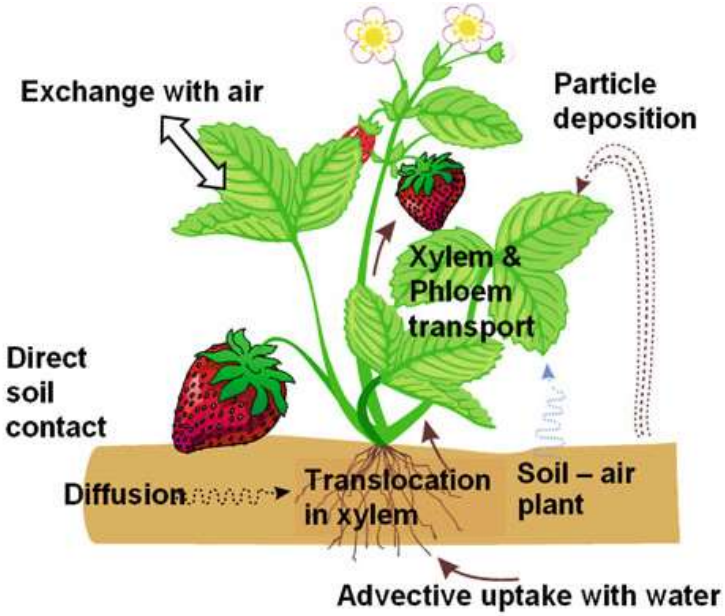


Figure 2.4: Transport and uptake processes in the soil-air-plant system (Trapp and Legind, 2011)

Pesticides applied/sprayed onto fruit and vegetables gather on the outer peel of the skin. However, pesticides may penetrate into the flesh since most of the fruits and vegetables skin do not have an impermeable barrier or membrane. Also, some pesticides are intended to absorbed into the tissue of the fruit or vegetable, which helps protect against the pest that infiltrates the skin to suck out the juices inside (Rabin, 2017). However, fruit or vegetables with thicker skin (such as cucumber and apples) are capable of keeping pesticides out of the flesh. Pesticides accumulations in fruit and vegetables can also result from transported pesticides by either rain, wind, or air from their point of application to the neighbouring crops (Rabin, 2017).

2.8 Physical parameters of triazines and Maximum residue limit (MRL)

2.8.1 Physicochemical parameters

The mobility of triazines in the environment is dependent on their physicochemical parameters including density, melting point, water-solubility, polarity, octanol-water coefficient (Log K_{ow}), lipophilicity, vapour pressure, and dissociation constant, etc. Triazines are known as weak bases due to their low pKa values, they have relatively low density compared to water, they are nonvolatile, and can undergo photolysis (Gunasekara et al., 2007). They are slightly soluble in water with high octanol water coefficient (above 2.5 except simazine) and thus they

are expected to adsorb on soil (Kunene and Mahlambi, 2019) and thus be absorbed by fruits and vegetables. Triazines also have low vapor pressure and thus they are unlikely to be present in air. Some of the physicochemical properties of triazines are shown in Table 2.1.

Table 2.1: Physicochemical properties of the triazines pesticides

Triazines	Density (g/mL) at 20°C	Melting Point (°C)	Water-solubility (mg/L) at 20°C)	Dissociation constant (pKa)	Lipophilicity value (LogP)	XLogP3	Vapour pressure (mm Hg) at 25 °C
Simazine	1.302 [a]	225–227 [a]	6.2 [a]	1.70 [b]	2.18 [c]	2.1 [a]	$6.08x10^{-6}$ [a]
Atrazine	1.23 [d]	173-175 [d]	34.7 [d]	1.68 [d]	2.61 [e]	2.3-2.71 [d]	$2.89x10^{-7}$ [d]
Ametryn	1.18 [g]	88-89 [g]	185 [f]	4.0 [f]	2.98 [g]	3.07 [f]	$2.74x10^{-6}$ [g]
Propazine	1.162 [h]	213 [h]	8.6 [h]	1.70 [h]	2.93 [h]	2.93 [i]	$1.31x10^{-7}$ [h]
Terbuthylazine	1.122 [j]	178 [j]	5.0 [j]	2.0 [j]	3.21 [j]	3.40 [j]	$1.12x10^{-6}$ [j]

[a] Aslam et al. (2013).

[b] Gunasekara et al. (2007).

[c] National Center for Biotechnology Information (2021).

[d] Atrazine. (2011).

[e] National Center for Biotechnology Information (2021).

[f] Shattar et al. (2017).

[g] National Center for Biotechnology Information (2021).

[h] Ronka et al. (2014).

[i] Paschke et al. (2004).

[j] National Center for Biotechnology Information (2021).

2.8.2 Maximum residues Limits (MRLs)

Residues are traces of pesticides left on the treated products or animals by veterinary drugs. The maximum residue limits (MRLs) are concentration levels that represent the maximum admissible concentration of each pesticide that a human can consume in the food crop without resulting in any negative effect (Lesueur et al., 2008). These standards must be legally tolerated or agree with the food protocol enforced by the government agricultural sector on food or feed when pesticides are applied correctly during agricultural practices (Bakırcı et al., 2014). The MRLs legislation was established by the USA, European Union (EU), and other countries to ensure safety by regulating pesticides in food products. They assist in ensuring that there is a proper use of pesticides via approval or legislation and registration (application rates and pre-harvest time intervals) and permit the free circular movement of pesticide-treated products (Bakırcı et al., 2014). The Environmental Protection Agency (EPA) and European Union (EU) priorities MRLs of the triazine pesticides for human and animal feed are indicated in Table 2.2.

Table 2.2: Maximum residue limit (MRL) values of triazines for food or feed, established by European Union (EU) and Japan legislation (Pasdar et al., 2017)

Triazine	Maximum residue limit (µg/kg)
Simazine	200
Atrazine	50
Ametryn	200
Propazine	250
Terbuthylazine	50

2.9 Extraction and detection techniques

2.9.1 Extraction techniques

Extraction techniques are used for chemical separation prior analysis. For the extraction of triazines in fruit and vegetables, some sample preparation techniques including Soxhlet extraction, microwave assisted extraction, ultrasonic extraction, solid-phase extraction (SPE), and QuEChERS have been used (Đurović & Đorđević, 2011). Soxhlet extraction is a routine procedure commonly used in many laboratories as it provides high recoveries (>60%). However, this technique requires usage of large solvent volumes (100–500 mL) and longer extraction times (6-24hours) which may result in analytes decomposition. Moreover, the filtration of the extracts as well as the additional clean-up step is required. Supercritical fluid extraction has been used as an alternative for Soxhlet extraction as it gives higher recoveries with reduced need of additional clean up step as the amount of interfering matrix in the extracts is lower. Microwave assisted extraction has also been used as a Soxhlet extraction alternative due to its shorter extraction time, lower solvent consumption, as well as the ability to concurrently analyse multiple samples. However, supercritical fluid technique needs an expensive apparatus, and also the microwave instrument is costly which limits their availability in many laboratories and thus their usage (Barchańska and Baranowska, 2009).

a) Solid-phase extraction (SPE)

The SPE was developed in the year 1971 as an alternative to the liquid separation technique. It is designed to separate suspended or dissolved solutes in a liquid or a solid mixture through the sorbent bed into desired and undesired substances. It combines pre-concentration and extraction abilities for organic compounds mixed with water by adsorption of a proper solid material and desorption with little amounts of organic solvents (Đurović & Đorđević, 2011; Żwir-Ferenc & Biziuk, 2006). It is very efficient in extraction when compared to liquid-liquid extraction as it uses a little solvent, hence decreased evaporation volumes of solvent during clean-up and reduced extraction time. The SPE is also used for the extraction of analytes, analyte pre-concentration and extract clean-up, it has high selectivity, greater reproducibility, and avoids emulsion formation (Żwir-Ferenc & Biziuk, 2006). It may also be used to prepare liquid samples, separate semi-volatile and non-volatile compounds from mixtures, and solids that have been pre-extracted into solvents.

The SPE involves four steps:

Step 1: conditioning - is where the sorbent functional groups are activated or conditioned with a solvent to prepare the sorbent bed. **Step 2: loading -** is where the solution containing the analyte is loaded and infiltrate through the solid phase and some impurities also remain in the sorbent. **Step 3: washing -** is where solvent (strong enough to remove impurities but not too strong to remove analytes of interest) is passed through the sorbent bed to eliminate co-extracts or impurities by washing them out. **Step 4: elution -** is where the analyte is eluted (with a small volume of solvent strong enough to completely remove the adsorbed analytes) down the column and collected for analysis. The four SPE steps are shown in Figure 2.5.

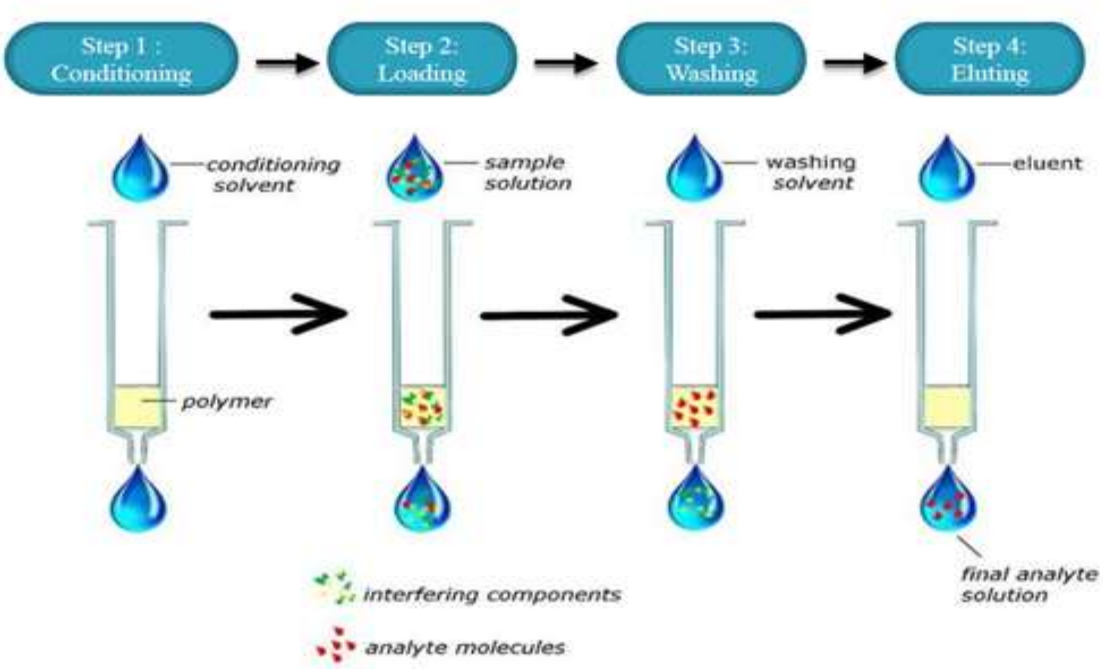


Figure 2.5: The steps involved in SPE technique (Sandoval Riofrio, 2017)

b) QuEChERS

The QuEChERS method is a quick, easy, cheap, effective, rugged, and safe method mainly designed for the extraction of analytes in fruits and vegetable samples. It consists of sample preparation and clean-up steps which increase the range of analytes recovered (Anastassiades et al., 2003). It is simple, accurate, has high recovery rates, and consumes low volume of solvent **(**Chembites, 2016).

Step 1: Sample preparation and extraction - the sample containing the analyte is homogenized uniformly (**A**). Extraction solvent (usually acetonitrile) is then added (**B**) followed by extraction salt (**C**). Salts, acids, or buffers might be required to enhance the extraction efficiency and to protect sensitive analytes. The buffer is employed in the AOAC 2007.01 method where it is used to stabilize sample pH by minimizing degradation of pH-sensitive pesticide residues. The mixture is then shaken/vortexed. An internal standard is added before vortexing to monitor and increase extraction efficiencies (**D**).

Step 2: Extract clean-up –A pipetted subsample of the solvent extract is cleaned up using dSPE (**D**), which is used to improve the QuEChERS technique by selectively absorbing organic acids, sugars, pigments, fatty acids, and other co-extractives through hydrogen interaction (Ji et al., 2008). Small polypropylene centrifuge tubes filled with $MgSO_4$ with precise weight and as well as PSA and C18 adsorbents are used to remove water that is in excess as well as the undesired components from the extracted samples. After shaking and separation through centrifugation, the cleaned extracts can be analysed using different techniques.

Step 3: Sample analysis – Sample pH needs to be adjusted most of the time to protect sensitive analytes and/or solvent-exchanged which will increase extraction efficiency and quantification (**E**) by either GC/MS or LC/MS. A simple QuEChERS extraction procedure diagram is shown in Figure 2.6.

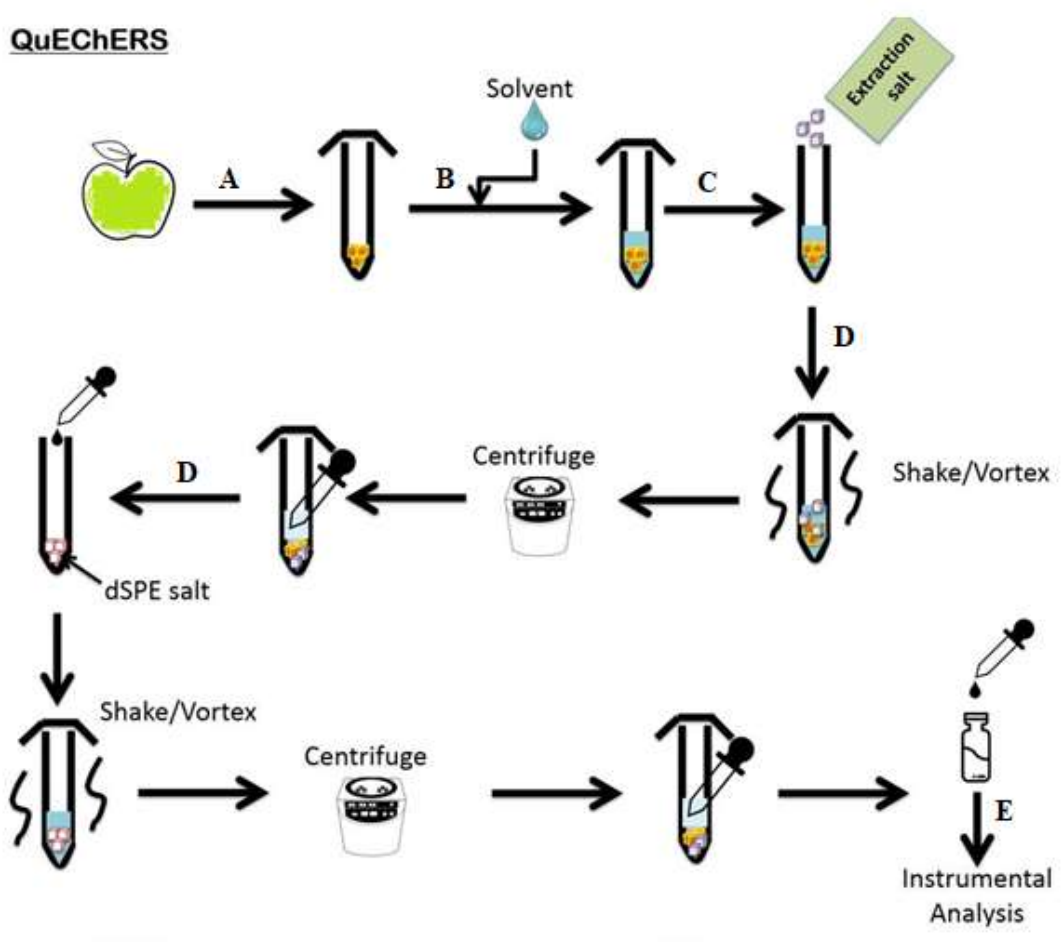


Figure 2.6: Flow chart of QuEChERS procedures (Chembites, 2016)

c) Ultrasonic Solvent Extraction (USE)

The USE method is an easy and more complete extraction method where the sample is immersed into a solvent within a vessel that is placed in an ultrasonication bath. This technique of sonication uses agitation which allows more intimate solid-liquid contact while the gentle heating contributes in speeding up the extraction process during sonication. Ultrasonication can also be used to maintain both pre-and post-harvest quality in terms of nutrients in fresh fruit and vegetables. It uses ultrasound at low temperatures to avoid sample degradation and loss of thermolabile constituents during the extraction (Cazes, 2004). This technique is very cheap, less time-consuming, uses low temperature, and yields good results. Solid-phase extraction can follow ultrasonic solvent extraction for extracts clean up. A typical example

(Figure 2.7) of an ultrasonic solvent extraction (USE) schematic showing the analysis setup:

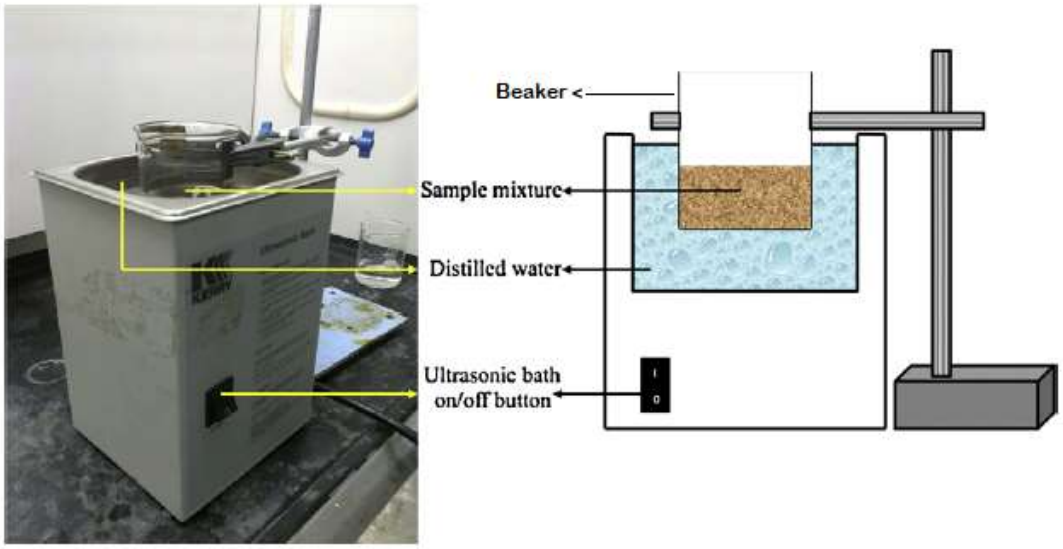


Figure 2.7: Ultrasonic Solvent Extraction (USE) experimental setup (Cheok et al., 2013)

2.9.2 Separation and detection techniques

Gas chromatography and liquid chromatography normally paired with mass spectrometry detectors are widely used for the analysis of pesticide residues in food matrices. Most pesticides are thermally unstable or non-volatile therefore, liquid chromatography is preferred. The LC-MS offers a powerful instrument for the determination of these compounds in food samples which are present at very low concentrations (Ortelli et al., 2004).

a) Liquid chromatography mass spectrometry (LC-MS)

Liquid chromatography-mass spectrometry can be used for thermal fragile and non-volatile molecules and can give both two- and three-dimensional data because of its detector. The LC-MS works by separating the mixture according to their physical and chemical properties, followed by the identification of the components within each peak and then the detection follows according to their mass spectrum. Thus, to increase the detection sensitivity for the completion of ionization the flow rate must be lower (Hird et al., 2014). A commonly adopted ionization is an electrospray ionization technique (ESI) that is applied for the determination of contaminants in food which is very good compared to the atmospheric pressure chemical ionization (APCI). This is because ESI can be used for highly polar, least volatile, or thermally unstable compounds. The APCI is normally chosen since it does not produce charged ions and can be used to analyse thermally stable polar and nonpolar substances (Lee et al., 2015).

The significant disadvantage that impacts quantification in liquid chromatography coupled to a mass spectroscopy detector (LC-MS) that uses an electrospray ionization (ESI) is the ion suppression/enhancement that is called matrix effect. This happens when the undesired components co-elute with the target compounds in the chromatographic separation (Hird et al., 2014). In the ESI ionic species are transferred from the mixture (liquid) into the gas phase, and involved three processes such as the spreading of fine spray droplets of charged droplets, solvent evaporation, and the injection of ions from the highly charged droplets (Ho et al., 2003). Therefore, the matrix effect's or undesired co-eluted components tend to compete for access to the surface of droplets and the following ion evaporation or property change in the eluent (i.e. volatility, surface tension) (Hird et al., 2014).

b) Gas Chromatography

The GC-MS is a method known to separate or analyze smaller and volatile molecules that are thermally stable in a mixture by a gas chromatography first where the sample is volatized. The volatized sample in a gas phase is then separated into many components via a capillary column packed in a stationary phase. An inert gas such as argon or helium or nitrogen is used to push the compounds and these components separate and elute at different retention times (Stashenko and Martínez, 2014). Advantages of a GC-MS is its ability to separate analytes in a complex mixture, measure analytes and can determine very low concentrations of organic pollutants. An ionization by the mass spectrometry of the separated component follows using electron or chemical ionization sources. In the mass analyzer (quadrupole or ion trap) ions are enhanced and they separation by their mass-to-charge (m/z) ratio. Then analysis and detection of compounds follow, showing a peak with height representing the quantity according to their m/z ratios (Stashenko and Martínez, 2014).

Mass spectrometry is used with both the gas and liquid chromatographic technique. It is a useful analytical method used to quantify and identify known and unknown compounds within a sample respectively and can be used in structural elucidation and chemical properties of various types of molecules (Kaklamanos et al., 2020). The MS works by converting the sample into gaseous ions then separating them into fragments or no fragments that can be quantified by the mass-to-charge ratio (m/z) and relative abundances of each ion type. Mass spectrometers comprised of three parts or components including ion source (gaseous ions are produced), mass analyzer (ions sorting and characterization to their mass components), and detector system (recording each ion's relative abundances), (Kaklamanos et al., 2020).

Due the high costs of MS which makes it to be unavailable in many laboratories, the ultraviolet detectors are often used with liquid chromatography, while flame ionisation and electron capture detectors are used with gas chromatography.

Absorbance Detector (UV/Vis) is the most common detector in LC and can measure the ability of solutes or effluent to absorb light at single or multiple wavelengths (da Silveira Petruci et al., 2017). The UV-Vis absorbance detectors are mainly used to detect any compounds absorbing at the wavelength monitored and can be used for gradient elution as well. The sensitivity of this detector depends on the molar absorption coefficient, the larger the value, the bigger the detector response. The UV-Vis absorbance detectors have a limit of detection of 10^{-8} M and a linear range of 10^{5}-fold range (da Silveira Petruci et al., 2017). There are three types of UV-Vis absorbance detectors which include fixed wavelength, variable, and diode array detectors (DAD or PDA).

Fixed wavelength detectors are cheap and simple detectors used in HPLC system (Swartz, 2010). The most common one utilized only 254 nm output wavelength from a low-pressure mercury lamp. Fixed wavelength detectors have a flexible and compact design that can offer a single variable wavelength range (190-750 nm) and is mostly a considered detector for the LC application in the detection of biological samples.

Variable wavelength detector is highly sensitive and provides stable baselines for the HPLC application since it offers multiple and variable wavelength detection. In a variable wavelength detector, up to four different wavelengths can be monitored simultaneously while gathering spectral data within the range of 190-750 nm because of its high scanning speed design which makes it easy to track and monitor impurities. The variable wavelength detector may be programmed or set to adjust wavelengths during a chromatographic analysis to make up for different analytes detection or set to a maximum absorbance of an analyte or a wavelength that enhances analyte selectivity. In a variable wavelength detector, light from a UV deuterium or tungsten (for visible) lamp is directed through a slit to a diffracting grating, which magnifies the light through its constituent wavelengths, allowing analytes to be detected. The diffracting grating moves or rotates to project a single wavelength of light through a slit, then to a photodiode through a detector/flow cell.

The photodiode array detector (PDA) operates or has an optical path similar to a variable wavelength detector excepts that the light from the lamp is passed through a detector/flow cell

before reaching the diffracting grating which then spread the spectrum of wavelength across the array of photodiodes. The PDA detectors can be used as multi-wavelength UV/Vis detectors and are very useful in method developments since they can provide a spectrum of elution peaks that can be used in peak or analyte identification or can be used to monitor co-eluting peaks (peak purity or homogeneity). The fixed wavelength and photodiode array detectors are illustrated in Figure 2.8.

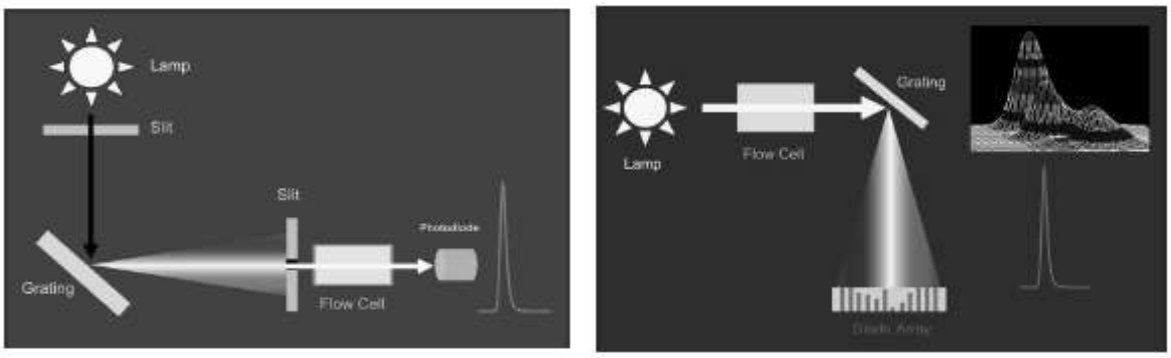


Figure 2.8: Illustration of a variable wavelength (a) and photodiode array (b) detectors (Swartz, 2010)

The flame ionization detector (FID) has a flame that is fuelled by hydrogen with an electrode located adjacent to it or near the air exit of the column. Thus carbon-containing compounds are pyrolyzed by the flame when they leave the column. During pyrolysis carbons can form electrons and cations which can generate a current between the electrodes, thus the detector can only detect only carbons and hydrocarbons (Grob et al., 2004; Higson, 2004). The increase in current lead to the development of peaks in the chromatogram and FID are unable to generate ions from carbonyl-containing carbons which lead to lower detection limits (less than pictograms per second).

Electron capture detector (ECD) is used to measure the degree of electron caught using radioactive electrons (i.e. beta particles) thus detecting molecules with electronegative or withdrawing elements and other groups such as halogens, carbonyl, nitriles, nitro groups, and organometallics. Carrier gas that could be used includes nitrogen or 5% methane in argon which is then passed between two electrodes adjacent to the adjacent to the anode (negative electrode) located in a radioactive foil such as 63Ni at the end of the column (Grob et al., 2004; Higson, 2004). A Current is produced when beta particles emitted from radioactive foil collide with and ionizes with carrier gas to generate more ions. The detection response is generated by the decrease in current which results from the captured electrons from electronegative or withdrawing elements or functional groups (Grob et al., 2004; Higson, 2004).

2.10 Analysis of pesticides in fruits and vegetables

Table 2.3: The summary of detection of pesticides in fruit and vegetable samples

Analyte	Sample	Extraction methods and detection	Extraction solvent	Comment	References
32 representative pesticides	Apple–blueberry sauce, peas and limes	QuEChERS - Original un-buffered, AOAC Official Method 2007.01 (acetate buffering), and European Committee for Standardization (CEN) Standard Method EN 15662 (citrate buffering). LC–MS/MS and GC–MS	Acetonitrile (MeCN) containing 0.1% acetic acid (HOAc), toluene, and ethyl acetate (EtOAc)	98% recovery was obtained for all three methods. This study suggested that a cheaper and green method within the three QuEChERS sample preparation methods can be utilized as sample preparation before triazine herbicides determination using an LC-PDA for analysis	Lehotay et al., 2010
Atrazine, simazine, propazine, ametryn,	Luffa, broad bean, and grape)	Dispersive solid-phase extraction (dispersive-SPE) coupled to liquid	Methanol and acetonitrile	80-110% recovery was obtained. The method was able to quantify all samples to be below the MRLs	Ji et al., 2008

prometryn, and prometon		chromatography-mass spectrometry (LC-MS)			
Ametryn, atrazine, cyanazine, prometryn, propazine, simazine, simetryn, terbuthylazine, and terbutryn	Drinking water	SPE (with oasis hlb sorbent) and HPLC-DAD	Acetonitrile, methanol, and acetone	Average recovery of 86% was obtained LODs were all below the MRLs and RSD was below 20%	Beceiro-González et al., 2014
Monocrotophos, dimethoate, imidacloprid, carbendazim, carbaryl and simazine	Leafy vegetables	Ultrasonic solvent extraction (USE) and liquid chromatography-tandem mass spectrometry (LC-MS-MS)	40 mL of ethyl acetate and 35 minutes sonication time	83.7-97.9% recovery was obtained LODs were all below the MRLs and RSD was below 3%	Pan et al., 2008
Atrazine, simazine,	Cherry tomato,	Ionic liquid (IL)-calixarene coated		71.5–96.9% recovery was obtained	Tian et al., 2014

ametryn, and cyanazine	strawberry, cucumber, garlic sprout, cole, cabbage, and tomato	solid-phase microextraction (SPME), sol–gel method, and gas chromatography–flame ionisation detector (GC–FID)		The developed method was found to be effective for the determination of triazines in fruit and vegetable samples	
Organochlorine pesticides	Small sized goldfish (Carassius auratus),	Ultrasonication with single-drop micro-extrcation (U-SDME) and GC/MS	Chloroform, n-hexane, methanol, and deionized water	82.1-95.3% recovery was obtained The method was found to be rapid, selective, sensitive and low cost for the determination of OCPs in fish. It could also be used useful for the health risk assessments and toxicokinetic studies in human beings and aquatic biota.	Shrivas and Wu, 2008
Ametryn, atrazine, cyanazine,	Dried edible seaweed,	Matrix Solid Phase Dispersion (MSPD), Solid Phase	n-Hexane, methanol, acetonitrile, ethyl	75-100% recovery was obtained The LODs and LOQs were acceptable and allowed the	Rodríguez-González et al., 2014

prometryn, propazine, simazine, simetryn, terbuthylazine and terbutryn)	sea lettuce (Ulva Lactuca), Wakame (Undaria pinnatifida), and Nori (Porphyrau mbilicalis),	Extraction (SPE), and HPLC-DAD	acetate, and Milli-Q water	determination of these compounds at the levels required by the legislation of seaweed for human consumption.	
Atrazine, malathion, fenitrothion, and parathion	Apple juices	Solid phase extraction (SPE), reversed-phase high-performance liquid chromatography (RP-HPLC) method with ultraviolet–diode array detection (UV-DAD)	Ultrapure water and acetonitrile	94.2–117.2 % recovery was obatined The developed method was declared suitable for the routine determination of investigated pesticides in apple juice samples	Velkoska-Markovska and Petanovska-Ilievska, 2013.
186 pesticides	1423 samples of fresh fruit	QuEChERS, ultrahigh performance liquid	acetonitrile (MeCN), glacial acetic acid	73 - 115%was obtained 48% of the	Bakırcı et al., 2014

	and vegetables	chromatography coupled with tandem mass spectrometry (UPLC/MS/ MS), gas chromatography with an electron capture detector (GC–ECD) and gas chromatography with mass spectrometry (GC–MS)	(HOAc), methanol, and deionized water	fruit samples and 83% of the vegetable samples contained pesticide residues above MRLs.	

2.11 References

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Chapter Three – Research Methodology

3. Experimental

3.1 Chemicals, standards, and analytical reagents

The triazine herbicide standards which includes ametryn (98.5%), atrazine (97.4%), propazine (99.3%), simazine (98.7%), and terbuthylazine (98.6%) were acquired from Sigma Aldrich (Riedel-de-Haen, Germany). HPLC grade analytical solvents; dichloromethane (99.9%), acetonitrile (99.9%), acetic acid (99.8%), acetone (99.8%), ethyl acetate (99.8%) and methanol (99.9%) were purchased from Sigma Aldrich and supplied by Honeywell (Steinheim, Germany). Sodium hydroxide pellets (97%) and sodium chloride (99%) were bought from Merck (Durban, South Africa).

3.2 Instrumentation

The solid phase extraction (SPE) vacuum manifold purchased from Sigma Aldrich (Steinheim, Germany) was connected to a vacuum pump from Edwards (Munich, Germany) and used for the extraction and pre-concentration of triazine herbicides in fruits and vegetables. The Oasis hydrophilic liphophilic balance (HLB), (60 mg, 3 mL) used as SPE sorbents were bought from Waters (Uppsala, Sweden). The roQ QuEChERS extraction kit and Phenomenex - roQ QuEChERS dSPE kit used for the extraction of triazine compounds fruits and vegetables were bought from Separations (Johannesburg, South Africa). The Buchi rotavapor R114 purchased from Labotec (Flawil, Switzerland) was used to concentrate the extracts. Ultrasonic bath used for the extraction of triazine herbicides from fruits and vegetables was bought from Science Tech (Durban, South Africa). The working frequency, power and temperature range of the ultrasonic bath was kept at 28 kHz, 300 W, and 45^{o}C respectively. The hand blender mixer used to homogenize samples was purchased from Clicks (Pietermaritzburg, KwaZulu-Natal). The centrifuge purchased from Shalom Laboratory (Durban, South Africa) was used for separation of supernatant liquid from solid. The analysis was performed using a liquid chromatography (LC-2020) fitted with Shim-Pack GIST C18-HP column (4.6 x 150 mm, 3µm) and LC-2030/2040 photodiode array detector (PDA) purchased from Shimadzu (Tokyo, Japan). The detector wavelength was set at 222 nm, the injection volume of 10 µL and flow rate of 0.65 mL/min were employed. The LC gradient program was used with a mobile phase composition of 0–3 minutes (48–52%, acetonitrile: water) and 3–25 minutes (30–70%, acetonitrile: water).

3.3 Standards preparation

A composite stock solution (100 mg/L) of triazine herbicides was prepared by mixing 1.0 mg of each analyte (triazines) into 10 mL of acetonitrile. The calibration curve and the calibration of the LC-PDA instrument were deduced from a series of working standard solutions (0.1 -1.0 mg/L), which were used for the quantification of triazine herbicides. The Stock solution and working standard solutions were kept in a refrigerator during the experiment.

3.4 Sampling

The selected fruits and vegetables samples were purchased from local middle and upper markets in Pietermaritzburg, KwaZulu Natal, South Africa. These included apples, pears, plums (pome fruits), carrots, potatoes, beetroot (root vegetables), tomatoes, avocados, cucumbers, bell pepper (fruiting vegetables), spinach, cabbage (leafy vegetables), peas (seed vegetables), bananas, (stem fruit), oranges, lemon (citrus fruit), passionfruit (exotic fruits), and grapes (flowing fruits).

3.5 Sample preparation and clean up

3.5.1 QuEChERS sample preparation

The fruits or vegetable samples were homogenized with the hand blender. Then 15 g of homogenized sample was accurately weighed into a clean 50 mL centrifuge tube and 15 mL of acetonitrile was added. The mixture was then fortified at 0.1 mg/kg of the triazine standard solution. Then a salt packet containing 6 g MgSO4 and 1.5 g NaOAc was added into the 50 mL centrifuge tube containing the mixture and was shaken for 1 minute vigorously. The mixture was centrifuged at 4000 rpm for 5 minutes, (Lehotay *et al*., 2010).

dSPE clean-up

The 8 mL of the supernatant from the above step was transferred into a 15 mL dSPE centrifuge tube containing 150 mg PSA and 150 mg C18. The tube was then shaken 30 seconds robustly by hand and centrifuged for 5 minutes at 4000 rpm to separate solid material from the liquid. A 1 mL of the supernatant (liquid) was transferred to an auto-sampler vial and 10 µL was injected into the LC-PDA for further analysis (Lehotay *et al*., 2010). All the analysis was done in triplicates.

a) Optimization of QuEChERS method

The QuEChERS method reported by Lehotay *et al* (2010) was adopted and further optimized to improve the extraction efficiency of all five triazine compounds. The QuEChERS parameters that were optimized included the effect of sample pH (5, 7 and 9), type of extraction solvents (acetonitrile, methanol/acetonitrile (50:50), and acetic acid/acetonitrile (1:99)) using a potato sample fortified at 0.1 mg/kg.

3.5.2 Sample preparation prior solid-phase extraction (SPE)

A 5 g of homogenized sample fortified at 0.1 mg/kg was accurately weighed into a 15 mL centrifuge tube and then 10 mL acetonitrile was added. The centrifuge tube containing the sample was vortexed for 2 minutes, then 2.5 g of NaCl was added and further vortexed for another 2 minutes. Then the mixture was centrifuged at 3000 rpm for 5 minutes, and then 5 mL of liquid (supernatant) was transferred into a 50 mL round bottom flask and allowed to evaporate at 35°C to almost dryness using a rotary evaporator. The extract was re-dissolved with distilled water to make up 100 mL in a volumetric flask and the extract was clean-up using an SPE (Yang *et al*., 2011).

Solid-phase extraction clean-up

Solid-phase extraction (SPE) was carried out using oasis HLB (60 mg, 3 mL) as the sorbent. The sorbent functional groups were conditioned with 6 mL of methanol to allow effective interaction with the analytes. Thereafter, 100 mL of sample solution was loaded. The sorbent was then washed with 3 mL of distilled water to remove impurities or interfering substances and then t dried for 10 minutes under vacuum to remove retained water. A 7.5 mL of methanol was used to elute the adsorbed analytes. The eluates were evaporated to 1 mL using a nitrogen evaporator and then subjected to an LC-PDA for further analysis (Kunene and Mahlambi, 2019). All the analysis was done in triplicates.

a) Optimization of SPE

The SPE method reported by Kunene and Mahlambi (2019) was adopted and further optimized to improve the extraction efficiency of all five triazine compounds. The SPE conditions that were optimized included the effect of sample pH (5, 7 and 9) and a loading volume of the sample (25, 50 and 100 mL) using a potato sample fortified with 0.1 mg/kg. SPE parameters such as the conditioning, washing, and eluting solvents were kept constant during the analysis.

3.5.3 Ultrasonic solvent extraction (USE)

A 5 g of homogenized sample fortified at 0.1 mg/kg was accurately weighed to a 100 mL beaker, then, 8 g of anhydrous sodium sulphate (Na_2SO_4) and 30 mL of ethyl acetate was added. Thereafter, the beaker was placed in the ultrasonic bath with the level of water above the level of the solvent inside the beaker and allowed to sonicate for 30 minutes. The ultrasonic bath temperature was allowed to rise from 45^{o}C at 28 kHz frequency and 300 W of power. After the sonication, the extract was filtered with a vacuum filter and the solid residue was rinsed with 2x10 mL volume parts of ethyl acetate. The filtered liquid was evaporated to dryness with nitrogen gas. Then the extract was re-dissolved with a 1 mL mixture of methanol:water with a composition of 40:60 (v/v) and then was filtered to an auto-sampler vial and then 10 µL was injected into the LC-PDA for further analysis (Pan et al., 2008). Some of the samples were subjected to a clean-up procedure using the SPE method before LC-PDA analysis to assess the effect of the clean-up procedure. All the analysis was done in triplicates.

a) Optimization of USE

The USE method published by Pan et al (2008) was adopted and further optimized to improve extraction efficiency of all the selected triazines. The USE extraction conditions optimized were the extraction solvents (acetonitrile, acetic acid, acetone, ethyl acetate, and methanol), the volume of extraction solvent (20, 30 and 40 mL), extraction time (15, 30 and 45 minutes), and different spike concentrations (0.05, 0.1 and1.0 ppm) Good extraction procedure should involve the solvent that better trap and dissolve the analytes making its liquid layer to be enriched with analytes of interest at reasonable extraction time with minimal solvent usage.

3.6 Methods validation

The optimized method was validated by evaluating the accuracy which was calculated as percentage analyte recovery from fortified samples and precision which was calculated as relative standard deviation (%RSD). Calibration curves were determined by plotting the peak area versus concentration of each analyte at 0.1 - 1.0 mg/kg concentration levels (Li *et al*., 2012). The linearity was evaluated from the calibration curve as correlation coefficients (R^2). The limits of detection (LOD) and limit of quantification (LOQ) which was determined by spiking of the blank sample with the lowest concentration achieved in the acceptable analyte recovery range (70-120 %) and precision (RSD $\leq$20 %). The LOD and LOQ were expressed as the analyte concentration corresponding to 3 and 10 times the standard deviation, respectively.

This was determined in accordance to the European Commission (EC) (2019) document no. SANTE/12682/2019.

3.7 Application to real samples

The QuEChERS-LC-PDA, SPE-LC-PDA, and USE-LC-PDA methods were then applied to real samples of food matrices (fruits and vegetables) after optimization and validation.

3.8 References

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Chapter 4 – Results and Discussion

4 Brief Introduction

It is worth noting that the results obtained from this work produced two papers where one paper has been published while the second paper has been sent to peer reviewed journal for possible publication.

Paper 1: HM. Mnyandu, PN. Mahlambi. Optimization and application of QuEChERS and SPE methods followed by LC-PDA for the determination of triazines residues in fruits and vegetables from Pietermaritzburg local supermarkets. Food Chemistry 360 (2021) 129818. https://doi.org/10.1016/j.foodchem.2021.129818.

Paper 2: HM Mnyandu, PN Mahlambi. Determination of triazines residues in fruits and vegetables: methods comparison of ultrasonic solvent extraction with and without solid phase clean-up (Submitted to Food Quality and Preference Journal).

The results have also been presented in a research symposium:

H.M Mnyandu, P.N. Mahlambi. QuEChERS method development and application for triazine herbicides determination in fruits and vegetables. PRIS Postgraduate Research and Innovation Symposium, 17 October 2019, University of KwaZulu-Natal, Westville (South Africa), Poster presentation.

Paper 1:

Optimization and application of QuEChERS and SPE methods followed by LC-PDA for the determination of triazines residues in fruits and vegetables from Pietermaritzburg local supermarkets

4.1 Abstract

QuEChERS and solid phase extraction (SPE) methods were optimized and applied for the extraction of triazines in fruit and vegetables. These extraction methods are easy, effective, rugged and safe. Also, they have the ability to purify the extracts which leads to lower detection limits and higher recoveries of the analytes. The analysis was conducted using liquid chromatography coupled to photodiode array detector. The limits of detection and quantification ranged from 0.4 -1.4 µg/kg and 1.5 – 4.5 µg/kg, respectively, for QuEChERS and 0.3 – 1.8 µg/kg and 1.4 – 4.9 µg/kg respectively, for SPE. The recoveries ranged from 84-102% for QuEChERS and 76-119% for SPE, with relative standard deviation less than 20% for both methods. The fruits and vegetables analysed were apples, pears, carrots, potatoes, tomatoes, avocado, cucumber, spinach, bananas, and oranges. The concentrations detected ranged between 6 – 46 µg/kg in fruits and 4 – 84 µg/kg in vegetables. Simazine was detected in all fruits and vegetable samples except in pear, while terbutylazine was not detected in all samples analysed. Propazine and ametryn were only found in carrot while pear sample only had atrazine. The proposed methods proved to be sensitive and accurate indicating their applicability for detection and monitoring of the selected triazines in fruits and vegetables. However, QuEChERS can be recommended for routine analysis of these triazines due to its fewer extraction steps compared to SPE which is important for turn-around time.

Keywords: Triazine herbicides, QuEChERS, solid phase extraction, liquid chromatography, photodiode array, fruit, and vegetables.

4.2 Introduction

Triazine pesticides are used to control or kill weeds and annual grasses in railways roadside and in various agricultural crops thus improving crops quality and yield, (Beceiro-González et al., 2014). Triazines tend to inhibit photosynthesis and therefore, kill the unwanted plants, even-though they should only be toxic to plants, they can also be toxic to human (Beceiro-González et al., 2014). The exposure to triazines have acute effect on humans such as eye irritation, dermal problems, headache, nausea, as well as the chronic effect such as cancer, birth

defect, interruption of hormone functions and the disruption endocrine system (Bakırcı et al., 2014; Fang et al., 2014).

Due to the extensive usage of triazines in agriculture, their residues can accumulate in food crops such as fruits and vegetables and can also be found in processed product such as fruit juice. This is because triazines can be absorbed from the soil by the root hairs, they can penetrate through the stomata or cuticle of the leaves and be translocated by phloem thus can reach fruit or vegetable produce. Triazines may also adsorb to the surface/skin of the fruit or vegetable and eventually penetrate into the flesh of the fruit or vegetable (Ando, 2019). It is therefore important that the triazines residues on or in food crops are monitored to ensure that they do not exceed their maximum residue limit (MRL), (Beceiro-González et al., 2014). The MRL for simazine and ametryn is 200 µg/kg, atrazine and terbuthylazine is 50 µg/kg and propazine is 250 µg/kg (Pasdar et al., 2017).

The characteristic matrix complexity of fruits and vegetables makes the extraction and cleanup the most problematic step in the determination of triazines in food stuffs. Moreover, these compounds are present in low concentration levels in fruits and vegetables which further challenge their direct determination using chromatographic methods. Hence, sample preparation is of paramount importance for their extraction and preconcentration prior the chromatographic determination (Pasdar et al., 2017). There are various sample preparation techniques that have been used for solid samples extraction which include supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), Soxhlet, ultrasonic extraction and microwave-assisted extraction. However, these techniques need long extraction time and consumes large volume of hazardous organic solvents, moreover they require additional time for extract clean-up. Furthermore, SFE and PLE techniques could require expensive instruments, which limits their availability in common analytical laboratories (Pasdar et al., 2017; Barchan´ska and Baranowska, 2009; Lang and Wai, 2001). Hence, ultrasound assisted extraction coupled to dispersive liquid-liquid microextraction solidified floating organic drop (UAE-DLLME-SFO) method has been developed and applied for extraction of triazines in fruits and vegetables. This extraction method is rapid as a result of high surface between the phases and the very small amount of solvent employed makes it environmentally friendly (Pasdar et al., 2017). Also, technique such as QuEChERS has been widely used as it is an environmentally friendly method that is simple and quick (Tadessea et al, 2016).

This work therefore focused on qualitative and quantitative analysis of triazine herbicides in fruit and vegetables (apples, pears; tomatoes, avocados; carrots, potatoes, spinach, bananas, cucumbers, and oranges) from Pietermaritzburg local markets. These fruits and vegetables were investigated as they are common in our diet, also they can be consumed raw or cooked, peeled or unpeeled hence the effect of the variables such as washed versus unwashed, peeled versus unpeeled as well as boiled fruit and vegetables was examined. The triazines (simazine, atrazine, ametryn, propazine and terbuthylazine) were extracted using QuEChERS and solid phase extraction followed by liquid chromatography coupled to photodiode array (LC-PDA) for the analysis. These triazines were chosen as there was a possibility for them to be present since they have been detected in river water, wastewater, soil and sediment samples from Pietermaritzburg where the vegetables and fruits were obtained (Kunene and Mahlambi 2019, 2020; Ntombela and Mahlambi, 2019).

The QuEChERS (AOAC 2007.01) which is a modified method used was followed by extract clean up using dispersive solid phase extraction (dSPE) containing C18 as the sorbent and primary secondary amine (PSA). The C18 helps on the removal of lipids, while PSA efficiently remove organic acid and saccharides. The modified QuEChERS method is one of the widely used as it involves the addition of anhydrous $MgSO_4$ and NaCl which aids on the separation and partitioning of the analytes into the organic phase. Moreover, the removal of acetonitrile from the aqueous phase and partitioning of polar analytes into acetonitrile is enhanced by addition of NaCl. QuEChERS method is also simple, has high efficiency and involves minimal number of steps (Łozowicka et al., 2017). SPE has been used due to its high sensitivity and effectiveness to extract, clean-up and pre-concentration of trace amounts of pesticides from various sample matrices. Even though mass spectrometry is more sensitive and selective (Covaciu et al., 2017), it is also costy which limits its usage. Hence, the analysis of LC coupled with a photodiode array detector is recommended for the determination of triazines due to their moderate polarity as well as their strong absorbance at approximately 220 nm line which results in the appropriate detection limit (Kunene and Mahlambi, 2019, 2020). The methods were both optimized to ensure their validity before application to real samples. To the best of our knowledge this work assess for the first time the concentration levels of the selected triazines in the selected local markets.

4.3 Experimental

4.3.1 Materials

The triazine herbicides standards, simazine (98.7%), atrazine (97.4%), ametryn (98.5%), propazine (99.3%) and terbuthylazine (98.6%) were purchased from Sigma Aldrich (Riedel-de-Haen, Germany). All HPLC grade solvents used; acetonitrile (99.9%), acetic acid (99.8%), and methanol (99.9%) were purchased from Sigma Aldrich and supplied by Honeywell (Steinheim, Germany). Sodium hydroxide pellets (97%) and sodium chloride (99%) were purchased from Merck (Durban, South Africa). The roQ QuEChERS extraction kit and Phenomenex - roQ QuEChERS dSPE kit were purchased from Separations (Johannesburg, South Africa).

4.3.2 Instrumentation

The Liquid Chromatography (LC-2020) fitted with Shim-Pack GIST C18-HP column (4.6 x 150 mm, 3µm) and LC-2030/2040 photodiode array detector (PDA) used for the monitoring of triazines from fruit and vegetable samples were purchased from Shimadzu (Tokyo, Japan). The detector wavelength set at 223 nm, the injection volume of 10 µL and flow rate of 0.65 mL/min were employed. The LC gradient program used was 0–3 minutes (48–52%, acetonitrile: water) and 3–25 (30–70%, acetonitrile: water).

The SPE vacuum manifold purchased from Sigma Aldrich (Steinheim, Germany) was connected to a vacuum pump from Edwards (Munich, Germany) and used for the extraction of triazines in fruits and vegetables. The Oasis hydrophilic liphophilic balance (HLB), (60 mg, 3 mL) used as SPE sorbents were purchased from Biotage (Uppsala, Sweden). The Buchi rotavapor R114 purchased from Labotec (Flawil, Switzerland) was used to concentrate the extracts. The Hand blender mixer used to comminute samples (to ensure sample homogeneity) was purchased from Clicks (Pietermaritzburg, KwaZulu-Natal). The centrifuge purchased from Shalom Laboratory (Durban, South Africa) was used for separation of supernatant liquid from solid.

4.3.3 Standards preparation

A 100 mg/L stock solution containing a mixture of triazines was prepared by dissolving 1 mg of individual standards in 10 mL acetonitrile. A series of six working solution (0.1 -1.0 mg/L) were prepared from the stock solution and used to calibrate the LC-PDA instrument. Five calibration graphs were constructed under optimum conditions to represent each of the analytes of interest. The standard solutions were stored in a refrigerator.

4.3.4 Sampling

The fruit and vegetable samples were purchased from local middle and upper markets in Pietermaritzburg (KZN), South Africa. These included apples, pears (pome fruits), carrots, potatoes (root vegetables), tomatoes, avocados, cucumbers (fruiting vegetables), spinach, (leafy vegetable), bananas (stem fruit), and oranges (citrus fruit). Four of each fruit/vegetable types were purchased weekly, kept in the refrigerator and used within three days.

4.3.5 Sample preparation and clean up

4.3.5.1 QuEChERS sample preparation

To comminute fruit or vegetable samples, a hand blender was used to ensure sample homogeneity. Then 15 g of homogenized sample was accurately weighed into a clean 50 mL centrifuge tube and mixed with 15 mL of acetonitrile and was spiked with triazine standard solution to make a concentration of 100 µg/kg. This was followed by dispensing the contents of the salt packet containing 6 g $MgSO_4$ and 1.5 g NaOAc into the 50 mL centrifuge tube containing homogenized sample. The centrifuge tube was then shaken vigorously by hand for 1 minute, then centrifuged for 5 minutes at 4000 rpm, making sure that the solid material is at the bottom of the tube and a supernatant (liquid) layer forms on top of the solid material (Lehotay et al., 2010).

dSPE clean-up

The supernatant liquid (8 mL) from the above step was transferred into a 15 mL dSPE centrifuge tube containing 150 mg PSA and 150 mg C18. The tube was shaken vigorously by hand for 30 seconds. The dSPE tube was then centrifuge for 5 minutes at 4000 rpm to separate solid material from the liquid layer. The supernatant liquid was transferred to an auto-sampler vial and analyzed via LC-PDA method (Lehotay et al., 2010).

4.3.5.2 Solid liquid sample extraction prior solid phase extraction clean-up

Fruit and vegetable samples were homogenised using a hand blender. Then 5 g of homogenized sample was accurately weighed into a 15 mL centrifuge tube and mixed with 10 mL acetonitrile. The mixture was vortexed for 2 minutes, then 2.5 g of NaCl was added and vortexed again for 2 minutes. The mixture was thereafter centrifuged for 5 minutes at 3000 rpm, and then 5 mL of the supernatant was transferred into a 50 mL round bottom flask which was placed in a rotary evaporator and evaporated at 35°C to almost dryness. The analytes were then re-dissolved with distilled water to make up 100 mL in a volumetric flask and was subjected to SPE for clean-up (Yang et al., 2011).

Solid phase extraction clean-up:

Solid phase extraction (SPE) was carried out using Oasis HLB cartridges (60 mg, 3 mL). The sorbent was activated/conditioning using 6 mL of methanol and then 100 mL of sample solution was loaded. The sorbent was then washed with 3 mL of distilled water to remove impurities and thereafter was allowed to dry under vacuum for 10 minutes to remove retained water. The adsorbed analytes were eluted with 7.5 mL of methanol. The eluates were then reduced/re-concentrated to 1 mL using a nitrogen evaporator and analysed using LC-PDA (Kunene and Mahlambi, 2019).

4.3.6 Optimization of the analytical method

4.3.6.1 Optimization of LC-PDA method

The LC-PDA method published by Kunene and Mahlambi (2019) was used with further modification. The mobile phase composition was optimized to improve the retention times of the analytes.

4.3.6.2 Optimization of QuEChERS method

The QuEChERS method published by Lehotay *et al* (2010) was adopted and further optimized to improve the efficiency of the extraction method. The extraction conditions optimized were the effect of pH (5, 7, and 9), extraction solvents (acetonitrile, methanol/acetonitrile (50:50), and acetic acid/acetonitrile (1:99)). The pH of 5 was achieved by only adding extraction salts packets containing $MgSO_4$ and NaOAc from the roQ QuEChERS extraction kit in the sample. While pH of 7 and 9 was achieved by adding extraction salts packets in the sample and adjusting the pH with NaOH.

4.3.6.3 Optimization of SPE method

The SPE method published by Kunene and Mahlambi (2019) was adopted and further optimized to improve the extraction efficiency of the method. The SPE parameters that optimized were the effect of pH (5, 7, and 9) and sample loading volume (25, 50 and 100 mL). The other SPE parameters which include conditioning, washing, and eluting solvents were kept constant during the analysis. The optimization of QuEChERS and SPE methods was conducted using a potato sample fortified with 100 µg/kg.

4.3.7 Analytical methods validation

The analytical methods were validated based on the limits of detection (LOD), limits of quantification (LOQ), linearity, percentage recoveries, and precision. Linearity was assessed by plotting the peak area against the concentration of each triazine compound and obtaining the calibration curve in concentration levels ranging between 0.1 - 1 mg/L (Li et al., 2012). The accuracy of the analytical method was validated using a potato sample fortified with 100 µg/kg mixture of triazine standards and was subjected to QuEChERS and SPE followed by LC-PDA for separation and quantification. Thereafter the percentage recoveries were calculated to evaluate the methods accuracy. The precision of the proposed methods was investigated by conducting all the analysis in triplicates. The LODs and LOQs were established by considering a signal-to-noise ratio of 3 and 10, respectively with reference to the background noise of the blank sample at the retention time of each triazine compound.

4.4 Results and discussions

4.4.1 Optimization of the LC-PDA

A gradient mode with a mobile phase composition was programmed as follows: 0-10 min (acetonitrile: water, 45:55%), 10-25 min (acetonitrile: water, 30:70%) with a detector wavelength of 223 nm and flow rate of 0.6 mL/min were initially used. Under these conditions, good separation of all triazines was achieved, however, the terbuthylazine peak was eluting at 23 minutes. To reduce the retention time, the program was then changed to 0-3 min (acetonitrile: water, 48:52%), 3-20 min (acetonitrile: water, 30:70%). Under these conditions the peaks were well separating and terbuthylazine was eluting at 17 minutes, and these were taken as the optimum conditions.

4.4.2 Optimization of QuEChERS method

a) Effect of sample pH on the extraction efficiency of QuEChERS

The effect of pH on the percentage recoveries of triazines was investigated using pH 5, 7, and 9. The extraction solvent acetonitrile was kept constant throughout the pH optimization step.

The percentage recoveries showed a decrease with an increase in pH and higher percentage recoveries ranging from 75-89% were achieved at pH 5 (Figure 4.1a). This could be due to that most of the triazines are basic sensitive and they degrade as the pH increases towards basicity (Zhao et al., 2011). Also, the pH of 5-5.5 is a compromise range between the quantitative extraction as well as the protection of alkali and acid-labile compounds (Łozowicka et al., 2017). The effectiveness of pH 5 could have been influenced by the addition of $MgSO_4$ (pH 5.5-6.5) and NaOAc (pH 5.2) in the sample without any further pH adjustment which increased

the chemical stability of all the triazines by absorbing water forcing them to be enriched in the acetonitrile layer thus improving extraction efficiency (Ji et al., 2008).

The t-test was then conducted in the mean recovery of pH 5 against the other pH results in order to investigate if they are significantly different. The obtained (table S1) p-values for pH 5 vs pH 7 ($p>0.006$), pH 5 vs pH 9 ($p>0.002$) are less than 0.05. This proved that there is a statistically difference between the mean values of the pH, therefore, a pH of 5 was chosen as the optimum pH.

b) Effect of extraction solvent on the extraction efficiency of QuEChERS

The influence of extraction solvent on the triazine percentage recoveries was examined using acetonitrile, mixture of acetic acid:acetonitrile (1:99) and a mixture of acetonitrile:methanol (50:50). The pH of 5 was kept constant throughout the extraction solvent optimization. Acetonitrile gave higher percentage recoveries ranging from 91-113% (Figure 4.1b). This could be due to that acetonitrile has higher chemical stability and a strong eluting strength than acetic acid and methanol (Yang et al., 2016). These results agree with those reported by Tankiewicz, (2019) where acetonitrile was considered as the solvent of choice for the extraction of pesticides residues in vegetables and fruits as it resulted to extracts with fewer interfering substances compared to acetone and ethylacetate. Moreover, acetonitrile is a preferred solvent when using QuEChERS method as it can be easily separated from water (salting out) (Tankiewicz, 2019). Acetonitrile was also found to be the best solvent compared to ethylacetate and acetone for the extraction of pesticides including triazines in soil using QuEChERS method (Łozowicka et al., 2017). Better extraction efficiency of acetonitrile was explained to be due to it high polarity compared to the other solvents used as the triazines are also polar. Ametryn showed lower interaction with the solvent which could be due to being more polar compared to the other selected triazine compounds because of it $–SCH_3$ substituent present in position 2 on the triazine structure than the –Cl substituent in other triazines (Rodríguez et al., 2013). However, its recovery was within the acceptable range of 70-120%. Similar observation has been reported by Ji *et al*., (2008) where highly polar triazines gave lower recoveries (<70%) with QuEChERS method due to the possibility of moving into the water layer during the extraction step.

The t-test results (table S2) also showed that the mean recovery result for acetonitrile is statistically different from mixture of acetic acid:acetonitrile and a mixture of acetonitrile:methanol extraction solvents as the p-values for acetonitrile vs acetic

acid:acetonitrile ($p>0.007$), acetonitrile vs acetonitrile:methanol ($p>0.05$) are less than 0.05. Acetonitrile was then selected as best extraction solvent.

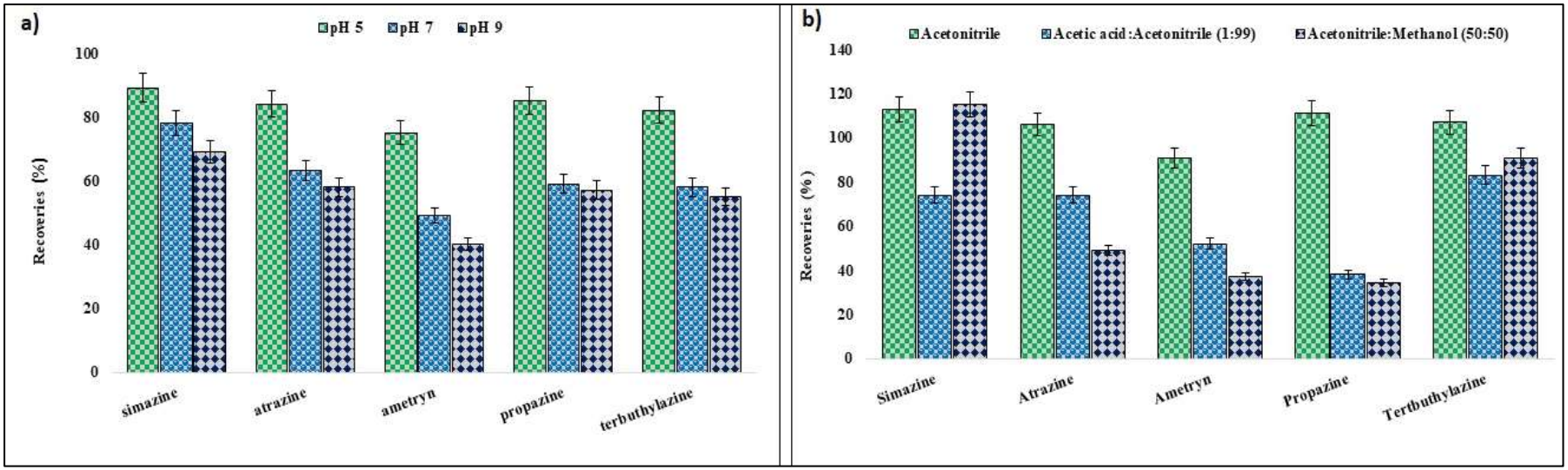

Figure 4.1: Effect of **a)** sample pH **b)** extraction solvent on triazines percentage recoveries (n=3), obtained using QuEChERS method

4.4.3 Optimization of the SPE method

a) Effect of sample pH on the extraction efficiency of SPE

The effect of pH was investigated since triazines are weak bases and they tend to protonate at excessively acidic pH conditions and hydrolyses at extremely basic pH conditions (Kunene, and Mahlambi, 2019). The effect of sample pH on the percentage recoveries of triazines from SPE was assessed using pH of 5, 7, and 9. The pH of 7 gave higher percentage recoveries ranging from 96-118% (Figure 4.2a). This could be due to the addition of NaCl (pH 7) which might have influenced the partitioning of these polar triazines into acetonitrile, thus improved their recoveries. Simazine had percentage recoveries $\geq$ 80% that was observed in all the pH values investigated. This could be due to its low water solubility compared to other triazines; hence it is easily extracted from the sample to the organic solvent resulting to it high adsorption by the sorbent bed (Kotrikla et al., 2006).

The t-test results (table S3) also revealed that the mean recovery result for pH 7 is statistically different from pH 5 and 9. The p-values obtained were ($p>0.0004$) for pH 5 vs pH 7, ($p>9.6x10^{-5}$) for pH 7 vs pH 9, ($p>0.01$) are less than 0.05, which confirmed that pH 7 is the better.

The pH of 7 was taken as the ideal pH**.**

b) Effect of sample loading volume on the extraction efficiency of SPE

The sample loading volumes of the sample (25, 50, and 100 mL) were studied to examine their effect on the percentage recoveries of triazines. Percentage recoveries > 70% were observed in all sample volumes, however, 100 mL gave the highest percentage recoveries (103-115%), (Figure 4.2b). This could indicate that there was a large amount of analytes available to interact and be adsorbed by the sorbent. Prior to SPE clean up step, the samples were re-concentrated by evaporation to almost dryness and then re-dissolved to the desired volumes (25, 50, 100 mL) and subjected to SPE. Therefore, for some of the compounds, 25 mL have higher percentage recoveries compared to 50 mL sample volume which could be due to low dilution factor in 25 mL than in 50 mL water.

The t-test results (table S4) displayed the mean recovery result for 100 mL to be statistically different from 25 and 50 mL sample volume with the p-value ($p>0.0007$) for 25 mL vs 100 mL and ($p>0.020$) for 50 mL vs 100 mL which are less than 0.05. Even though some of the recoveries for 25 mL were higher than those in 50 mL, their p-value ($p>0.3$) is above 0.05 which implies that they are not statistically different. Therefore 100 mL volume was selected as the efficient sample loading volume.

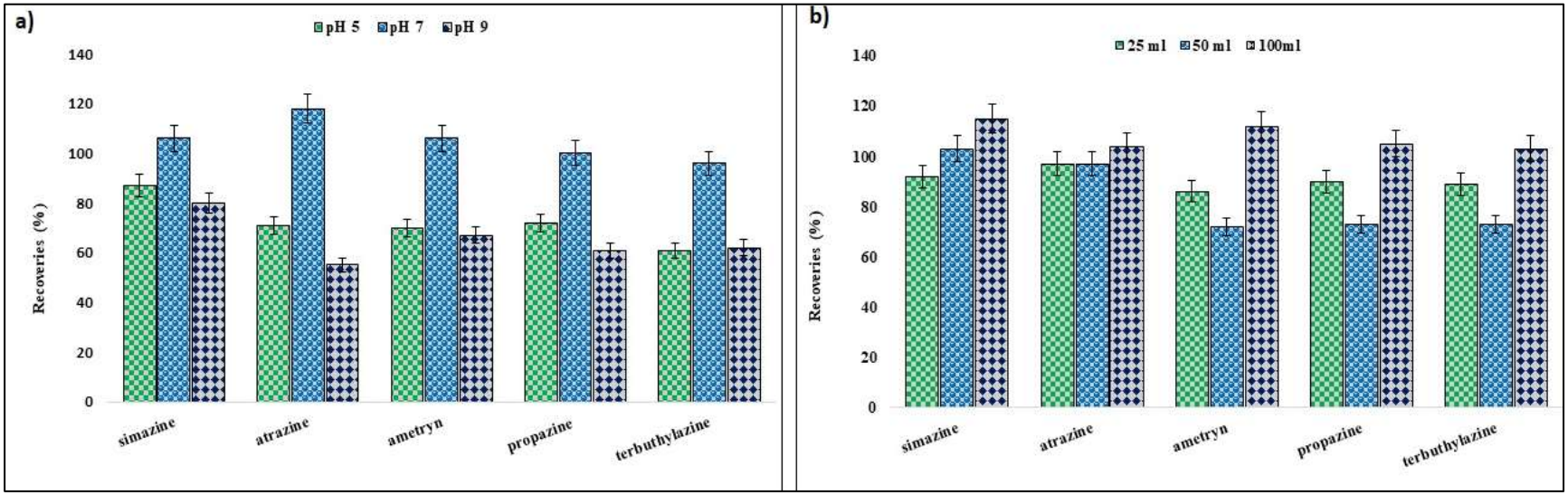

Figure 4.2: Effect of **a)** sample pH **b)** loading volume on triazines recoveries (n=3), obtained using SPE method

4.4.4 Analytical method validation

The method was validated in terms of linearity, LODs, LOQs, precision and percentage recoveries. Good linearity with correlation coefficient above 0.99 was observed for all triazines. The LODs and LOQs obtained ranged from 0.4 – 1.4 µg/kg and 1.5 – 4.5 µg/kg, respectively, for QuEChERS and ranged from 0.9 – 1.8 µg/kg and 2.4 – 4.9 µg/kg, respectively, for SPE (Table 4.1 and Table S5). The LODs and LOQs for both methods are lower than the maximum residue limit of the triazines which means that the proposed methods can be successfully employed for the determination of these triazines in real samples. Even-though, these results are comparable, they suggest that QuEChERS is slightly more sensitive than SPE. However, SPE is more sensitive towards atrazine compared to QuEChERS. The higher sensitivity of QuEChERS could be due its ability to eliminate matrix effect when it is applied to fruits and vegetables (Tankiewicz, 2019). The percentage recoveries ranged from 84-102% for QuEChERS and 100-111% for SPE which indicates that SPE is slightly more accurate compared to QuEChERS. Both methods had relative standard deviation (RSD) less than 20% which indicated good precision. These percentage recoveries for QuEChERS method are comparable to those obtained by Lehotay et al, (2010) and Rizzetti et al., (2016) where atrazine was recovered at 96%, and 97% respectively. Terbuthylazine was recovered (78%), simazine (82%) and ametryn (84%) recoveries were reported for QuEChERS method (Rizzetti et al., 2016), and terbutylazine (107%), (Cherta et al., 2013). The SPE recoveries obtained in this study are also in agreement with those reported in literature for atrazine (105%), (Cherta et al., 2013) and terbuthylazine (85%), (Rizzetti et al., 2016).

The t-test results confirmed that LOD and LOQ result for both methods are not statistically different from each with the p-value ($p>0.11$) and ($p>0.034$), respectively as they are higher than 0.05. Also, the recovery results for both methods were statistically proven to be significantly different with the p-value value ($p>0.02$) which is below 0.05. The performance for both methods is comparable and has been statistically confirmed not to be different. However, QuEChERS method can be recommended for routine analysis as it involves only two extraction steps which reduce the overall extraction, while SPE involves four steps hence it has more parameters that need to be validated. Also, QuEChERS uses lesser amount of solvent compared to SPE which makes it environmentally friendly.

Table 4.1: Correlation coefficient (R^2), LOD (µg/kg), LOQ (µg/kg), Recovery (%R) and RSD of the triazines in potato sample spiked at 100 µg/kg, (n=3) and MRLs (µg/kg), (Pasdar et al., 2017)

Pesticides	QuEChERS			SPE			MRL	R^2
	LOD	LOQ	%R ± RSD	LOD	LOQ	%R ± RSD		
Simazine	1.4	4.5	102 ± 11.3	1.8	4.9	111 ± 4.1	200	0.9979
Atrazine	1.1	3.8	96 ± 10.9	0.9	2.4	111 ± 6.3	50	0.9994
Ametryn	0.7	2.2	84 ± 9.6	1.0	2.8	109 ± 2.8	200	0.9978
Propazine	0.4	1.5	99 ± 13.2	1.2	3.0	103 ± 2.5	250	0.997
Terbuthylazine	0.6	1.8	95 ± 13.2	1.5	4.7	100 ± 3.5	50	0.9995

4.4.5 Application of the QuEChERS-LC-PDA method on fruits and vegetables

a) Effect of pre-treatment variables on the concentrations of triazines in fruits and vegetables from middle market.

The samples (carrot, tomato, potato, apple, and pear) were subjected to various pre-treatments such as washed versus unwashed, peeled versus unpeeled and well as 10 minutes boiling pre-treatment. Each sample was analyzed in triplicates to ensure the reliability of the results.

The unwashed + unpeeled pre-treatment showed the highest concentrations of triazines in all samples indicating that pesticides were adsorbed onto the vegetable's skin. Higher concentrations observed in potatoes and pears after peeling indicates that the pesticide has infiltrated in the fruit and vegetable. Also, pesticides are introduced in fruits and vegetables before blooming or during growth or after harvesting which may results in pesticides to be found in different locations in the same fruit or vegetable (Bajwa and Sandhu, 2011).

The lower residual levels observed after the boiling pre-treatment indicates that this thermal processing treatment results in different pesticides degradation (Bajwa and Sandhu, 2011). Even though the concentrations of triazines showed some decrease after the application of each pre-treatment, the t-test results showed that they are not statistically different from each other and p-values were ($p < 0.27$) unwashed + unpeeled vs washed + unpeeled, ($p < 0.11$) unwashed + unpeeled vs peeled. However, after the boiling pre-treatment a significant difference was observed with ($p < 0.022$) which is less than 0.05 for unwashed + unpeeled vs boiled.

Simazine was the most dominant compound found in all fruits and vegetables samples except for a pear samples which showed only atrazine (Table 4.2). This suggests that simazine was the excessively used pesticide in the farming of food crops. The maximum simazine residual concentration detected in the unwashed and unpeeled potatoes (34.3 µg/kg) is comparable to those obtained from a study that was conducted in Brazil which reported simazine present in unwashed and unpeeled potatoes with a concentration of 34.5 µg/kg (Pasdar et al., 2017). Atrazine was the second dominant triazine to be detected which could be due to that atrazine and simazine are often absorbed by plant roots. Also, atrazine is one of the most severely employed and less expensive pre and post emergent used against a broad spectrum of weeds on various crops (Sharma et al., 2017).

Carrot was the most contaminated sample and contained all the analysed triazines except terbuthylazine which was not detected in all samples. This is due to that carrots absorb

pesticides from soils and accumulates them in the tissue (Waliszewski et al., 2008; Covaciu et al., 2017). The triazine residual levels found in all the fruits and vegetables samples were all below MRLs (50 - 250 µg/kg), indicating that they are safe for consumption.

Table 4.2: Concentrations (µg/kg) of triazines obtained in potatoes, apples, carrots, pear, and tomatoes from local middle market, (n = 3)

Pre-treatment variable	Simazine	Atrazine	Ametryn	Propazine	Terbuthylazine
Unw + unp potato	34.3±4.5	nd	nd	nd	nd
W + unp potato	32.3±1.8	nd	nd	nd	nd
Peeled potato	34.2±7.1	nd	nd	nd	nd
Boiled potato	26.5±3.6	nd	nd	nd	nd
Unw + unp apple	10.8±1.4	nd	nd	nd	nd
W + unp apple	9.9±7.6	nd	nd	nd	nd
Peeled apple	6.9±2.5	nd	nd	nd	nd
Boiled apple	nd	nd	nd	nd	nd
Unw + unp carrot	32.7±12.5	30.8±10.1	64.3±2.3	71.8±6.5	nd
W + unp carrot	30.9±11.4	28.3±3.51	60±12.1	nd	nd
Peeled carrot	31.0±7.8	18.9±4.76	45.1±5.2	nd	nd
Boiled carrot	19.3±2.3	13.1±5.26	nd	nd	nd
Unw + unp pear	nd	18.5±7.90	nd	nd	nd
W + unp pear	nd	14.0±8.41	nd	nd	nd
Peeled pear	nd	20.5±6.53	nd	nd	nd
Boiled pear	nd	15.1±7.16	nd	nd	nd
Unw + unp tomatoes	34.3±5.2	nd	nd	nd	nd
W + unp tomatoes	31.8±4.9	nd	nd	nd	nd

Peeled tomatoes	27.5±6.2	nd	nd	nd	nd
Boiled tomatoes	22.0±2.0	nd	nd	nd	nd

Note: Unw + unp = unwashed + unpeeled; W + unp = washed + unpeeled; nd = not detected

b) Comparison of traizines concentrations detected in vegetables and fruits from middle and upper markets

The concentrations of triazines were compared in vegetables and fruits purchased from local middle and upper supermarkets (Table 4.3). Simazine was found to be present in all vegetable samples from both markets. This could be due to that simazine is an herbicide that is applied to control annual grasses and broadleaf weeds before seedlings are planted as a result of its excellent broadleaf weed control. However, germination or established vegetables may be affected by simazine residual if they are planted in the treated soil within 18 months of the treatment. Therefore, it is possible that trace levels of simazine can be transferred from the treated soil to the root vegetable. Also, pesticides disperse slowly after being sprayed on crops and need a waiting period before crop harvesting which differ in different crops, therefore higher residual levels may be expected in fruits or vegetables if harvested before the required waiting period is completed (Jallow et al., 2017).

Ametryn was present in carrots from both markets while propazine was found in carrots from middle market only. Carrots was found to be the most contaminated vegetable. The presence of ametryn could be due to its usage in controlling annual grasses and broad-leaves weeds. While the presence of propazine could be due to its application on carrots, celery and fennel applied as a spray during planting or immediately after planting but before weed growth (Pohanish, 2015). The concentrations of triazines in potatoes and carrots purchased from upper market were slightly lower compared to the middle market except for tomatoes where it is vice versa. The apples and pears from middle market were found to be contaminated with atrazine only which could be due its heavy usage as pre and post emergence weeds controller in apples (Sharma et al., 2017). The detected triazines residues in all vegetables and fruits purchased from both markets are below the maximum residue levels which make them safe for consumption. This also indicates that vegetables and fruits from upper markets are less susceptible towards human health effect. The presence of pesticides in organic carrots has been observed by (Chiarello and Moura, 2018). This has been associated with the possibility of

pesticides usage to alter the soil microbiome and hence could indirectly result in the formation of residues in organic carrots (Chiarello and Moura, 2018).

The presence of triazines in fruits and vegetables could also be due to the possible usage of effluent water from wastewater treatment plants for irrigation purpose and also the sludge as the fertilizer. The presence of triazines residues from effluent water or sludge may leach into the soil, be absorbed by crops and can end up being consumed by human beings. Also, the triazines are persistent in the soil, which consequently results in the long-term transfer of their residues from agricultural areas where they have been employed previously. These could be the possible reasons for the detection of triazines in upper market vegetables even though they are expected to be free of triazines as they sell organic vegetables.

Table 4.3: Comparison of concentrations (µg/kg) of triazines obtained in vegetables and fruits from local middle and upper markets, (n = 3)

Triazines	**Potatoes**		**Tomatoes**		**Carrots**		**Apples**		**Pears**	
	UM	MM	UM	MM	UM	MM	UM	MM	UM	MM
Simazine	31.9±5.5	34.3±4.5	39.5±2.3	34.3±5.2	22.2±4.9	32.7±12.5	nd	nd	nd	nd
Atrazine	nd	nd	nd	nd	nd	nd	nd	16.3±5.0	nd	20.1±1.9
Ametryn	nd	nd	nd	nd	36.1±5.4	64.3±2.3	nd	nd	nd	nd
Propazine	nd	nd	nd	nd	nd	71.8±6.5	nd	nd	nd	nd
Terbuthylazine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

UM – upper market, MM – middle market, & nd – not detected

c) Comparison of concentrations obtained in fruits and vegetables using QuEChERS and SPE method

To evaluate the efficiency of the two modified QuEChERS and SPE methods, similar samples were extracted using both methods and their concentrations were compared. The samples included spinach, avocado, banana, orange, and cucumber purchased from a local middle market. The results obtained showed that both methods are efficient at extracting these compounds. They extracted similar compounds in both fruits and vegetables with comparable concentrations for atrazine in avocado, simazine in cucumber and terbuthylazine in spinach (Table 4.4) which could be due to their comparable LOD and LOQ values. Furthermore, higher concentrations were observed for ametryn in banana and simazine in spinach using SPE compared to QuEChERS, while higher concentration was obtained for terbuthylazine in orange when QuEChERS was used. The higher concentrations observed for SPE could be due to that it is more accurate compared to QuEChERS. However, the t-test results approved that the concentrations obtained by both methods are not statistically different from each other with the p-value ($p>0.7$) which is higher than 0.05.

Simazine was detected in spinach (51 - 84 µg/kg) and cucumber (10 - 14 µg/kg) samples from both methods. This could be due to simazine's application as a pre-emergence and it can persist in the environment such as soils which make it easier to be absorb by the spinach roots, translocate and accumulate on the leaves leading to it high concentrations. The lower concentrations detected in cucumbers could be due to its thick skin which makes it not it easy for the pesticides to penetrate through. Atrazine was only present in avocado at very low concentrations (4.0 – 6.0 µg/kg) which could be due to greater matrix effect as avocado is rich in fat content. The matrix effect is interpreted as a suppression of detector signals leading to low peak area that is directly proportional to the concentration (Pano-Farias et al., 2015). Amertyn was only quantified in banana samples (19 - 38 µg/kg) which could be due to ametryn being used to kill broad-leaved weeds and annual grasses in bananas, sugarcane, maize, and pineapple fields (Ali et al., 2016). Terbuthylazine was obtained in spinach (17 - 20 µg/kg) and orange (23 - 46 µg/kg). The terbuthylazine concentration detected in citrus fruit (orange) is higher compared to the concentration (3 µg/kg) reported by Anastassiadou et al. (2020) using QuEChERS method.

Table 4.4: Concentrations (µg/kg) of triazines obtained in spinach, avocado, banana, orange, and cucumber samples from local middle market, (n = 3)

Triazines	**Spinach**		**Avocado**		**Banana**		**Orange**		**Cucumber**	
	SPE	QuEChERS	SPE	QuEChERS	SPE	QuEChERS	SPE	QuEChERS	SPE	QuEChERS
Simazine	84±3.3	51±5.8	nd	nd	nd	nd	nd	nd	10±6.8	14±5.0
Atrazine	nd	nd	6.0±2.7	4.0±5.5	nd	nd	nd	nd	nd	nd
Ametryne	nd	nd	nd	nd	38±6.5	19±10.9	nd	nd	nd	nd
Propazine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Terbuthylazine	20±2.1	17±7.2	nd	nd	nd	nd	23±4.0	46±11.4	nd	nd

nd = not detected

4.5 Conclusion

The applicability of the proposed multi-residue analysis methods (QuEChERS- and SPE-LC-PDA) showed to be comparable in qualitative and quantitative determination of five triazines in fruits and vegetables. The optimization of the methods improved their extraction efficiency and precision which is crucial to obtain reliable residue data as well as ensuring the quality of marketed fruits and vegetables. The pre-treatment showed to be the effective way of removing pesticides from the surface, however, it does not eliminate the systematic pesticides present inside the fruit or vegetable. Simazine was found to be the most dominant triazine in fruit and vegetable samples, while carrot was the most polluted vegetable as it contained most of the analysed triazines. All the samples analysed contained residues of triazines, however, they were all below the MRLs. Since there are limited studies on monitoring of triazines in South African fruits and vegetables, the results obtained in this work can be employed to increase the database of triazines in African produce. They may also be used to inform the policy makers about their remedial measures and create awareness.

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Chapter 5 – Results and Discussion

Paper 2:

Determination of triazines residues in fruits and vegetables: methods comparison of ultrasonic solvent extraction with and without solid phase clean-up

5.1 Abstract

An ultrasonic solvent extraction (USE) method has been optimized and applied with and without solid-phase clean-up for analysis of triazine herbicides (viz. simazine, atrazine, ametryn, propazine, and terbuthylazine) in fruits and vegetables. The determination of triazines was done using liquid chromatography paired with a photodiode array detector (LC-PDA). The methods showed recoveries ranging between 75 and 81% for USE with SPE clean-up, and 102 and 106% for USE without SPE clean-up and with RSD < 15% for both methods which implied that the methods were precise and accurate. The limit of detection (LOD) ranged from 1.1-1.8 µg/kg for USE with SPE clean-up and 0.6 – 1.0 µg/kg for USE without SPE clean-up. The limit of quantification (LOQ) was between 3.4 – 5.2 µg/kg for USE with SPE clean-up and 1.7 – 2.9 µg/kg for USE without SPE clean-up.

The USE with and without SPE clean-up methods was then applied to fruits (grapes, lemon, passionfruit, and plum), and vegetables (beetroot, bell pepper, cabbage, and peas) samples purchased from a local middle market. The concentration levels of triazines detected were beetroots (102 - 152 µg/kg), bell pepper (45 - 88 µg/kg), grape (14 - 22 µg/kg), lemon (3.2 - 156 µg/kg), passionfruit (32 - 222 µg/kg), and peas (126 – 138 µg/kg) and plum (9 – 11 µg/kg). All the triazines were quantified at levels below the MRLs in fruits and vegetables which reveals that they are not threatful to human health. Propazine was the most dominant followed by atrazine and then simazine, while terbuthylazine was not detected in all samples. The comparison of the methods revealed that USE has good sensitivity, selectivity, and efficiency at the small residual levels of triazines in fruits and vegetables and can be successfully applied without the additional SPE clean-up. Moreover, the proposed method can be considered an inexpensive, environmentally friendly method that can be used for daily monitoring of the concentration levels of the selected triazines in fruits and vegetables if they can be consumed without causing any adverse effect on humans.

Keywords: ultrasonic extraction, solid-phase extraction, fruits, vegetables, liquid chromatography

5.2 Introduction

Triazine pesticides are pre- and post-emergence herbicides used to control the growth of large-leaved weeds and thereby increase crop quality and yield. Triazines are chemically stable and very persistent in the environment, and because of their poor soil absorption, they can move through the soil and into water sources (surface and groundwater), (Tian et al., 2014). Also, from the soil, they may be absorbed by root plants and further translocate to different plant parts including the fruits and vegetables. Triazine traces from fruits and vegetables may end up being consumed by human beings resulting in adverse health effects including severe reproductive, developmental, immunological, and neurological impacts (Klementova and Keltnerova, 2015). Triazines are also associated with potential mutagenic and carcinogenic effects (Kunene and Mahlambi, 2020). Because of the toxicity of triazine pesticides, it is critical to establish responsive and precise analytical methods to monitor their levels in fruits and vegetables and assess the risk they pose to human health.

A Simple and cheap sample preparation technique such as ultrasonic solvent extraction (USE) is more useful and essential for the isolation of analytes from the sample (Shrivas, 2008). The USE employs ultrasound waves to pass through the solvent and produces cavitation thus enhances the extraction efficiency of the organic compounds. The application of ultrasound results in the production of cavitation bubbles which then compress and collapse as the pressure and temperature increase. The bubble collapse generates shock waves that pass through the solvent amplifying the mixing of the sample matrix and the extraction solvent (Barbero et al., 2008). Ultrasonic also has a mechanical effect that promotes good penetration of the solvent into the sample matrix, resulting in the increased contact surface area amongst the solid matrix and the liquid phase. This strengthens the mass transfer and disruption of cells through the collapsing of cavitation bubbles which releases the organic compounds into the solution. High temperature (>40^{o}C) increases the number of cavitation bubbles formed thus increasing the extraction efficiency of the USE method (Barbero et al., 2008). The extraction efficiency of USE to retrieve multi-residues can be improved through the optimization of the solvent type and extraction time (Pan et al., 2008; Rezić et al., 2005).

The application of solid-phase extraction (SPE) clean-up on USE extracts may reduce the co-extract interferences that are highly soluble in an organic solvent and can thus improve the chromatographic analysis. The SPE is a highly selective technique that can be divided into parts that include a liquid (sample matrix or solvent with analytes) and solid phase as sorbent.

The analytes can be extracted by adsorption from a liquid matrix onto a solid sorbent bed, which is followed by the elution of the analytes from the sorbent into an organic solvent that may be injected for chromatographic separation and detection. Thus, the application of USE followed by the SPE clean-up method promotes effective extraction and determination of triazine residues present at very low concentrations in fruit and vegetable samples (Pan et al., 2008).

A daily diet that incorporates a combination of fresh fruits and vegetables is important for human health promotion and protects protection against some of the deteriorating diseases such as cardiovascular disease, aging, brain dysfunction, and cancer, stabilize and control blood sugar levels and cholesterol levels (Mebdoua, 2019). However, consumption of fruits and vegetables containing traces of triazines can have health effects. Therefore, this work aimed to optimize USE for the extraction of triazine residues in fruits and vegetables to assess if their concentration levels are within the limits that are safe for human consumption. The comparison of USE extraction efficiency before and after the application of the solid phase extract clean-up was also investigated. The separation and quantification of the extracted triazines were conducted using liquid chromatography coupled with a photodiode array detector due to atrazine's strong absorbance at the 220 nm line, which provides good detection limits. Even though ultrasonic has been employed with and without the clean-up step, to the best of our knowledge, the comparison of these methods was conducted for the first time in this work. Also, its application on the selected fruits and vegetables from the local supermarket has not been reported, furthermore, not much assessment of South African fruits and vegetables has been reported.

5.3 Experimental

5.3.1 Chemicals and reagents

The standards ametryn (98.5%), atrazine (97.4%), propazine (99.3%), simazine (98.7%), and terbuthylazine (98.6%), and HPLC grade organic solvents which include acetone (≥99.8%), acetonitrile (≥99.9%), ethyl acetate (≥99.7%) and methanol (≥99.9%) were purchased from Sigma Aldrich (Durban, South Africa). Analytical grade anhydrous sodium sulphate (Na_2SO_4) was obtained from Merck (Durban, South Africa).

5.3.2 Samples and standard preparation

Fruits (grapes (berries), lemon (citrus), passionfruit (exotic), and plum (stone)), and vegetables (beetroot (root), bell pepper (fruiting), cabbage (leafy), and peas (seed) were purchased from local supermarkets in KwaZulu-Natal (PMB), South Africa. A stock solution of 100 ppm was prepared by dissolving 1.0 mg of each triazine standard with acetonitrile in a volumetric flask. A series of working standards (0.1 - 1.0 ppm) were prepared by diluting the stock solution in acetonitrile and were further used to calibrate the LC-PDA instrument.

5.3.3 Instrumentation

An ultrasonic bath used for the extraction of triazine in fruit and vegetable matrices was purchased from Science Tech (Durban, South Africa). Hand blender mixer used to homogenize samples were obtained from Clicks (Pietermaritzburg, South Africa). The SPE vacuum manifold was obtained from Sigma Aldrich (Steinheim, Germany), while a vacuum pump was from Edwards (Munich, Germany). Oasis Hydrophilic Lipophilic Balance (HLB) cartridges, (60 mg, 3 mL) were obtained from Biotage (Uppsala, Sweden). The LC-PDA used for triazines separation and quantitation analysis was purchased from Shimadzu (Tokyo, Japan). The LC-PDA consists of an LC-2020 fitted with LC-2030/2040 photodiode array detector (PDA), set at 222nm wavelength and was obtained from Germany. Chromatographic separation was performed on a Shim-Pack GIST C18-HP column (4.6 x 150 mm, 3µm), utilized as the stationary phase for LC-PDA. The extract injection volume and the flow rate were set at 10 µL and 0.65 mL/min respectively. An LC gradient program: 0–3 min (48–52%, acetonitrile: water) and 3–25 min (30–70%, acetonitrile: water) was employed.

5.3.4 Sample preparation

5.3.4.1 Ultrasonic Solvent Extraction (USE)

A 5 g of homogenized sample fortified with 0.1 mg/kg triazine standard mixture was accurately weighed to a 100 mL beaker, then, 8 g of anhydrous sodium sulphate (Na_2SO_4) and 30 mL of ethyl acetate was added. Thereafter, the beaker was placed in the ultrasonic bath with the level of water above the level of the solvent inside the beaker and was allowed to sonicate for 30 minutes. The ultrasonic bath temperature was set at 45°C at 28 kHz frequency and 300 W of power. After the sonication, the extract was filtered with a Whatman filter-paper (0.45µm) fitted to a funnel, and the solid residue was washed with 2x10 mL portions of ethyl acetate. The filtered liquid was evaporated to almost dryness with nitrogen gas and the extract was re-dissolved with a 1 mL mixture of methanol: water with a composition of 40:60 (v/v) and then was filtered to an auto-sampler vial using 0.2 µm acrodisc filters and then 10 µL was injected

into the LC-PDA for further analysis (Pan *et al*., 2008). Some of the samples were subjected to a clean-up procedure using the SPE method before LC-PDA analysis to assess the effect of the clean-up step. All the analyses were done in triplicates.

5.3.4.2 Optimization of USE method

The USE method published by Pan et al (2008) was adopted and further optimized to improve the extraction efficiency of all the selected triazines. Also, a good extraction procedure should involve the solvent that better trap and dissolve the analytes making its liquid layer to be enriched with analytes of interest at reasonable extraction time with minimal solvent usage.
The USE conditions optimized were the extraction solvents (acetonitrile, acetone, ethyl acetate, and methanol), volume of extraction solvent (20, 30 and 40 mL), and extraction time (15, 30 and 45 minutes).

5.3.4.3 Solid Phase Extraction (SPE) method

SPE method published by Mnyandu and Mahlambi (2021) was adopted for sample clean-up after ultrasonic solvent extraction before LC-PDA analysis. Methanol (2x3 mL) was used to condition the SPE cartridge (sorbent bed) and then 100 mL of sample was passed through the SPE cartridge. Thereafter, 3 mL of distilled water was used to wash the sorbent bed and the cartridge was allowed to dry under vacuum for 10 minutes to remove the retained water. The extract was eluted with 7.5 mL of methanol and was subjected to evaporation by nitrogen gas to 1 mL. The extract was then filtered to an auto-sampler vial using 0.2µm acrodisc filters and 10 µL of the filtered extract was injected into an LC-PDA for further analysis.

5.4 Analytical method validation

The accuracy, which was calculated as percentage analyte recovery from spiked samples, and precision, which was calculated as relative standard deviation (%RSD), were used to verify the optimized procedure. The linearity was evaluated from the calibration curve as correlation coefficients (R^2). The limits of detection (LOD) and limit of quantification (LOQ) were obtained by spiking the blank samples with the lowest concentration possible, achieved in the acceptable analyte recovery range (70-120 %) and precision (RSD $\leq$20 %). The LOD and LOQ were calculated by multiplying the analyte concentration by three and ten times the standard deviation, respectively. This was determined in accordance to the European Commission (EC) (2019) document no. SANTE/12682/2019.

5.5 Results and discussion

5.5.1 Optimization of USE method

a) Influence of extraction solvent on the USE extraction efficiency without SPE clean-up

Extraction solvents such as acetone, acetonitrile, ethyl acetate, and methanol were investigated for their influence on the extraction efficiency of triazines using USE method without SPE clean-up. The results obtained (Figure 5.1) showed that acetonitrile was highly efficient in extracting all triazines with %recovery ranging from 86-121%. This indicates that acetonitrile was more effective in absorbing and transmitting the energy of the ultrasound and thus enhanced the extraction efficiency of the ultrasonic extraction (Barbero et al., 2008). Also, acetonitrile is more polar compared to the other solvents employed, hence it was more efficient in solubilizing the atrazine and thus improved their transfer from the sample to the solvent. Also, polar solvents tend to increase the isolation of triazines and other pesticides during their extraction (Rodríguez et al., 2013). Therefore, acetonitrile was chosen as an optimum extraction solvent. The t-test results (Table S8) also showed that the mean recovery result for acetone, ethyl acetate, and methanol are not statistically different with $p>0.78$ for acetone vs ethyl acetate, $p>0.064$ for acetone vs methanol, $p>0.18$ for ethyl acetate vs methanol which is above 0.05. However, the mean recovery for acetonitrile is statistically different from acetone, methanol, and ethyl acetate. The p-values obtained were ($p>0.019$) for acetonitrile vs acetone, ($p>0.0060$) for acetonitrile vs ethyl acetate, ($p>0.044$) for acetonitrile vs methanol, are less than 0.05, which confirmed that acetonitrile is a better extraction solvent.

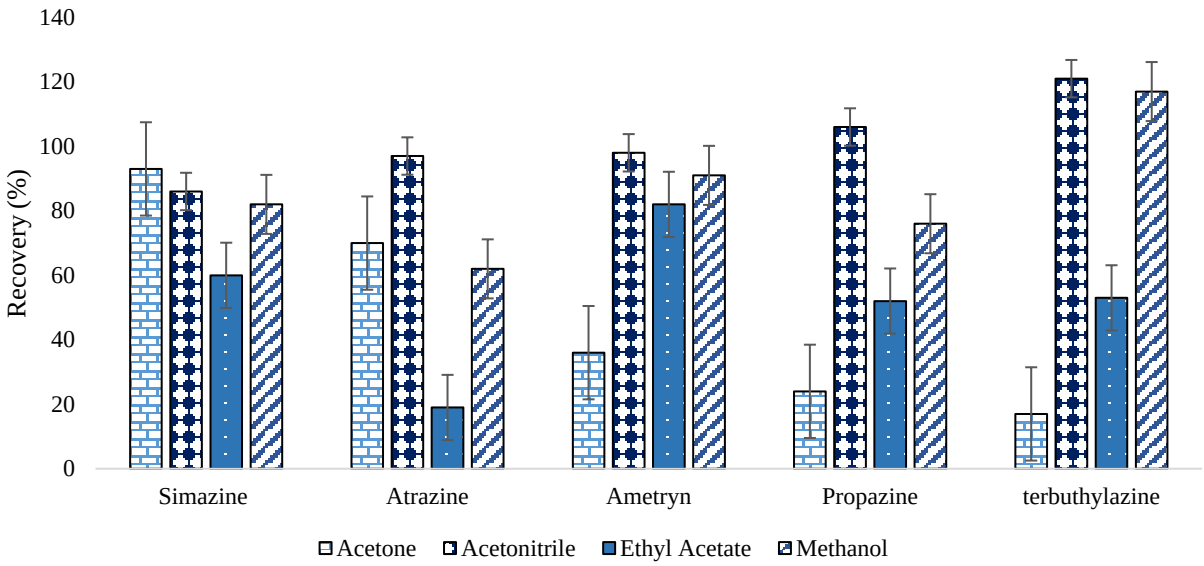

Figure 5.1: Influence of extraction solvent on the USE extraction efficiency using a potato sample fortified at 0.1 ppm

b) Influence of extraction solvent volume on the USE extraction efficiency without SPE clean up

The extraction solvent volume was investigated using 20 mL, 30 mL, and 40 mL of acetonitrile. The results (Figure 5.2) showed that triazines were highly recovered (86-121%) when 30 mL was used. The lower recoveries in 20 mL could indicate that this volume was not enough to allow the complete dissolution of the analytes, hence a lower quantity was recovered in the solvent. While at higher volume (40 mL), lower analytes recoveries obtained may be attributed to weak solvent-sample interaction (Kunene and Mahlambi 2020) or could be due to higher dilution of the analytes. Based on the t-test, the mean recovery result (Table S9) for 20 mL and 40 mL is not statistically different with the $p>0.38$ higher than 0.05. However, mean recovery for 30 mL is statistically different from 20 mL and 40 mL as the p-value obtained were $p>0.0096$ for 30 mL vs 20 mL, $p>0.0074$ for 30 mL vs 40 mL are less than 0.5 indicating that 30 mL is the suitable extraction solvent volume. The 30 mL solvent volume was chosen as an optimum.

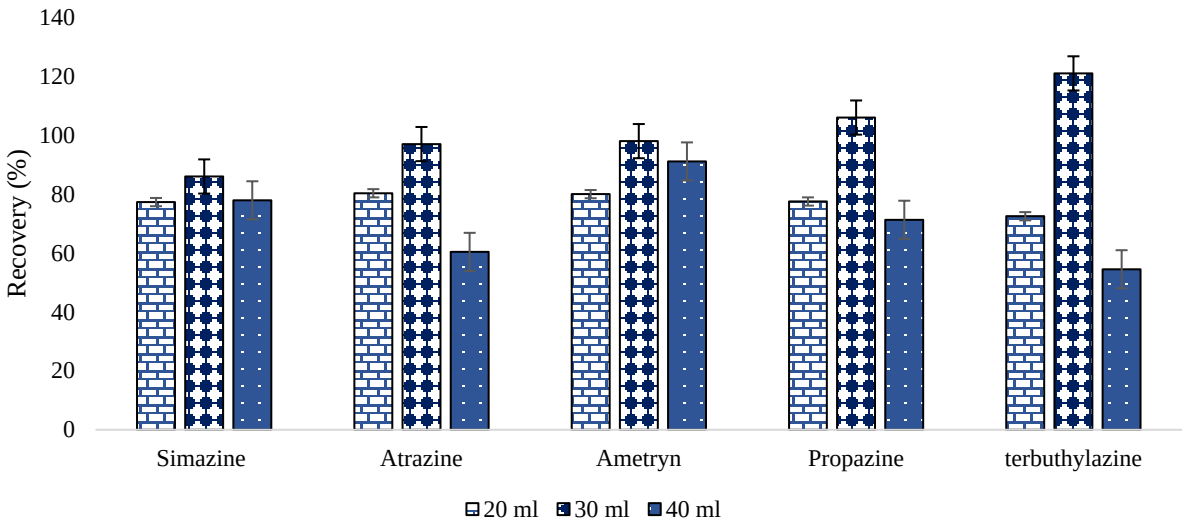

Figure 5.2: Influence of extraction solvent volume on the USE extraction efficiency using a potato sample fortified at 0.1 ppm

c) Influence of extraction time on the USE extraction efficiency without SPE clean up

The effect of extraction time was investigated using 15, 30, 45 minutes. The results (Figure 5.3) showed that extraction time of 30 minutes gave the highest recoveries for all the triazines (86-121%). This could be due to that increasing extraction time allows efficient contact between the analytes at the solvent and thus increases the amount of analytes being transferred to the organic solvent (Kunene and Mahlambi, 2020). However, further prolong of extraction time may result in the risk of analytes degradation which could be the reason for reduced recoveries at 45 minutes (Barbero et al., 2008). The lower recoveries at 15 minutes may indicate that the time allotted was insufficient to complete the extraction, and thus the analytes remained in the sample. The t-test results (Table S10) approved that the mean recovery result for 15 minutes and 45 minutes is not statistically different with the $p>0.28$ which is greater than 0.05. However, mean recovery for 30 minutes is statistically different from 15 minutes and 45 minutes as the p-value obtained were $p>0.05$ for 30 minutes vs 20 minutes, $p>0.0033$ for 30 minutes vs 40 minutes are less than 0.05 revealing that 30 minutes is an efficient extraction time. Hence, 30 minutes was selected as the ideal time.

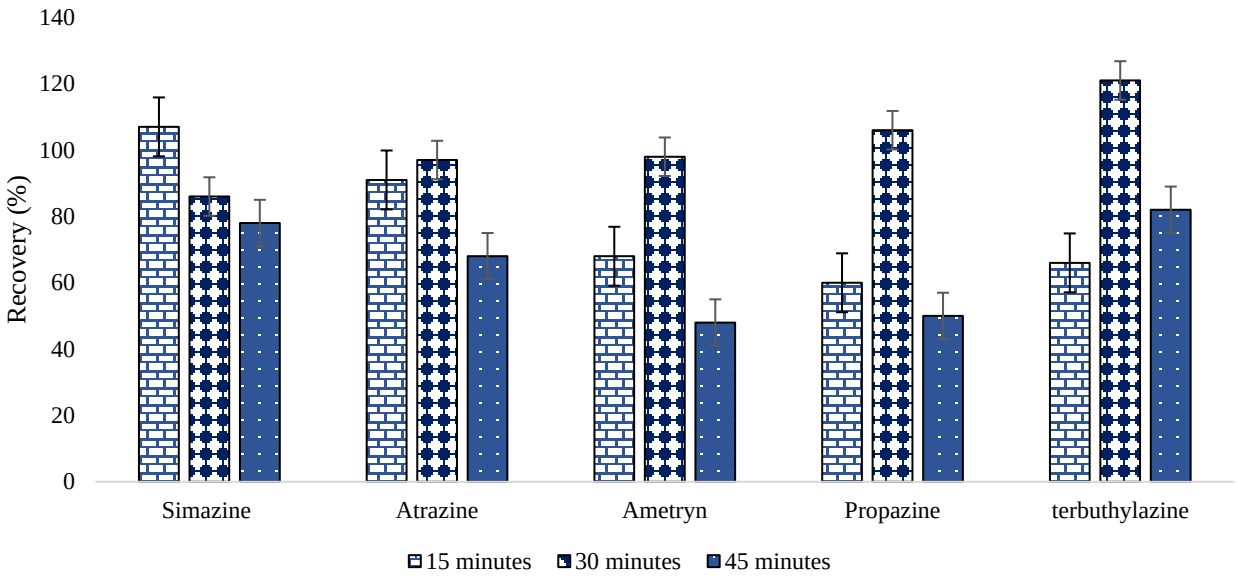

Figure 5.3: Influence of extraction time on the USE extraction efficiency using a potato sample fortified at 0.1 ppm

d) Influence of spiking with different concentrations on the USE extraction efficiency without SPE clean up

The influence of spike concentration on the extraction efficiency was examined using 0.05, 0.1, and 1.0 ppm. The results (Figure 5.4) showed that there is no major impact of spiking with different concentrations. This reveals that the extraction efficiency of the method is independent of the analyte concentration which is important as the concentration in real samples is not known. The t-test results (Table S11) also agreed that the mean recovery results are not statistically different with the $p>0.89$ for 0.05 ppm vs 0.1 ppm, $p>0.594$ for 0.05 ppm vs1.0 ppm, and $p>0.56$ for 0.10 ppm vs 1.0 ppm.

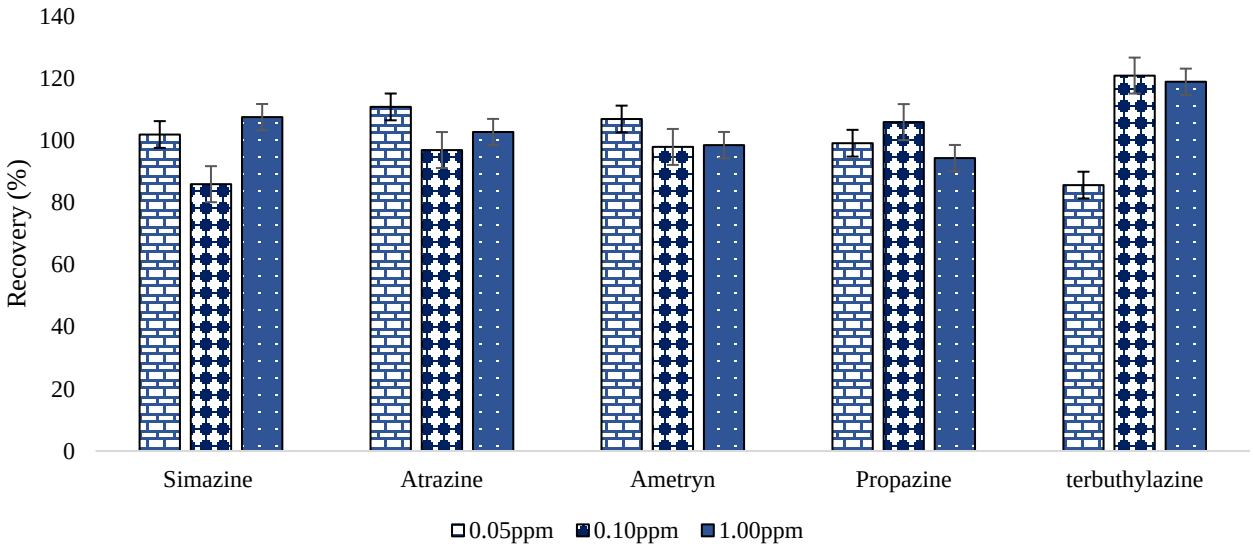

Figure 5.4: Influence of spiking with different concentration on the USE extraction efficiency using a potato sample fortified at 0.05, 0.1, and 1.0 ppm

5.5.2 Validation of the analytical method

The method validation was evaluated using the percentage recoveries, precision, linearity, LODs, and LOQs. The correlation coefficient (R^2) value greater than 0.99 was obtained for all triazines (Table 5.1 and Table S12a/b), implying good linearity of the method. The LODs and LOQs obtained for USE without SPE clean-up ranged from 0.6 - 1.0 µg/kg and 1.7 - 2.9 µg/kg respectively. While the LODs and LOQs obtained for USE method with SPE clean-up ranged from 1.1 - 1.8 µg/kg and 3.4 - 5.2 µg/kg. Both methods gave lower LODs and LOQs than the maximum residue limits indicating that they can be applicable for the analysis of trace levels of triazines in fruits and vegetables. Acceptable triazines recoveries ranging from 102 - 106% and 75 - 81% for USE with and without SPE clean-up, respectively were obtained. A relative standard deviation (RSD) of less than 20% was achieved for both methods. This indicates that USE without SPE clean-up is more accurate for the extraction of the selected triazines in fruits and vegetables. This could be due to many steps involved when the methods are combined resulting in a prolonged process carried under room temperature which might have promoted analytes degradation from the processes such as hydrolysis or microbial decomposition (Rodríguez-González et al., 2013). Moreover, the combination of extraction methods might have resulted in the loss of analytes due to over-extraction as the analytes move from the USE to SPE clean-up. The statistical analysis also confirmed that the LOD, LOQ, and recovery mean results for USE without SPE clean-up are statistically different from those of USE with SPE

clean-up. The p values were the $p>1.5x10^{-7}$ for recovery, p>0.0037 for LOD and p>0.0015 for LOQ results which are all below 0.5 signal significant difference between the mean results (Table S12a/b).

Table 5.1: Limit of detection (LOD) (µg/kg), limit of quantification (LOQ) (µg/kg), Recovery (%R), RSD of the triazines in potato sample spiked at 0.1 ppm, (n=3), MRLs (µg/kg), (Pasdar et al., 2017), and correlation coefficient (R^2), for the USE method with/without SPE clean-up

Pesticides	**USE**			**USE + SPE**			MRL	R^2
	LOD	LOQ	%R ± RSD	LOD	LOQ	%R ± RSD		
Simazine	0.7	2.1	105 ± 9.0	1.3	3.8	81 ± 4.1	200	0.9995
Atrazine	0.6	1.7	105 ± 8.5	1.1	3.4	79 ± 6.3	50	0.9996
Ametryn	0.7	2.2	105 ± 9.9	1.3	3.9	76 ± 2.8	200	0.9994
Propazine	0.7	2.2	106 ± 10.5	1.3	3.9	75 ± 2.5	250	0.9993
Terbuthylazine	1.0	2.9	102 ± 12.4	1.8	5.2	79 ± 3.5	50	0.9993

5.5.3 Application of the ultrasonic solvent extraction with and without solid phase clean-up to real fruits and vegetables.

a) Comparison of concentrations obtained in fruits and vegetables using USE method with and without SPE clean up.

The fruits (grapes, lemon, passionfruit, and plum), and vegetables (beetroot, bell pepper, cabbage, and peas) from a local middle-market were extracted using the USE method with and without SPE clean-up and their concentrations were compared to evaluate methods efficiency. The results obtained (Table 5.2) showed that both methods were able to extract triazine compounds efficiently which could be due to their high extraction efficiencies. The concentration levels obtained with USE without SPE clean are slightly higher compared to those obtained with SPE clean-up which could be due to the high accuracy and precision observed for USE without the clean-up.

Propazine was detected (14-222 µg/kg) in all the samples except cabbage and plum for both methods with the highest concentration in passion fruit. This could be due to propazine being a pre-emergence pesticide commonly applied directly to the soil and can also be absorbed through the leaves and roots within the plant. Propazine can also persist in the soil due to its moderate degradation under aerobic conditions with a half-life of 2-3 months and 3-6 months in loamy sand that is sterile and nonsterile, respectively (Maples, 2014). Atrazine was detected at 11-62 µg/kg in bell pepper, passion fruit, and plum with the highest concentration in bell pepper. Atrazine is very persistent in soil with a half-life of 26 to 142 days especially in areas where it has not been previously applied since atrazine is resistant to abiotic hydrolysis and to direct aqueous photolysis and is moderately prone to aerobic biodegradation (Maier & Gentry, 2015; Liu, 2014). Simazine was only detected in lemon and plum at low concentration levels (3.2 – 11 µg/kg) with the highest concentration in plum. The presence of simazine could be due to it use as an excellent pre-emergence in controlling broadleaf weeds fruits, berries, citrus, and nuts (Heri et al., 2008). Terbuthylazine was not detected in all samples analyzed while cabbage samples were uncontaminated by all the triazines analyzed. The presence of triazines in fruit and vegetable samples could be due to them being absorbed by the plants through contaminated soil or irrigation water. The concentration levels detected in all the triazine were below the maximum residue limits (MRLs) which suggest that these fruits and vegetables from local middle markets are safe for human consumption.

Table 5.2: Concentrations (µg/kg) of triazines obtained in fruits and vegetable samples from local middle market, (n = 3)

Triazines	**Beetroot**		**Bell Pepper**		**Cabbage**		**Peas**	
	USE	USE + SPE	USE	USE + SPE	USE	USE + SPE	USE	USE + SPE
Simazine	nd	nd	nd	nd	nd	nd	nd	nd
Atrazine	nd	nd	62 ± 5.6	45 ± 6.0	nd	nd	nd	nd
Ametryn	nd	nd	nd	nd	nd	nd	nd	nd
Propazine	154 ± 2.3	102 ± 2.1	88 ± 9.7	49 ± 6.3	nd	nd	138±2.2	126±4.7
Terbuthylazine	nd	nd	nd	nd	nd	nd	nd	nd
	Lemon		**Passionfruit**		**Grape**		**Plum**	
	USE	USE + SPE	USE	USE + SPE	USE	USE + SPE	USE	USE + SPE
Simazine	5.3 ± 7.8	3.2 ± 6.7	nd	nd	nd	nd	11 ± 3.1	9.9 ± 6.9
Atrazine	nd	nd	33 ± 2.8	32 ± 6.3	nd	nd	12 ± 1.9	11 ± 4.1
Ametryn	nd	nd	nd	nd	nd	nd	nd	nd
Propazine	156 ± 9.0	154 ± 10.6	222 ± 13.5	167 ± 11.9	22 ± 1.6	14±3.3	nd	nd
Terbuthylazine	nd	nd	nd	nd	nd	nd	nd	nd

5.6 Conclusion

In this study, a simple and easy, and environmentally friendly ultrasonic solvent extraction method was successfully optimized and applied with and without SPE clean-up for the determination of triazine residues in fruits and vegetables. The results showed that USE with or without SPE clean-up can be accurate for the determination of the selected triazines in the analyzed fruits and vegetables. Also, the LODs, LOQs obtained for both methods were below the MRL values indicating the applicability of the methods to extract trace levels of triazines in fruits and vegetables. However, USE without SPE clean-up showed to be more accurate and sensitive and hence can be recommended for effective daily monitoring of triazines in fruits and vegetables.

Propazine was found to be the most dominant triazine in fruits and vegetables with higher concentrations quantified that range from 14 – 222 µg/kg and its highest concentration was found in passion fruits. Cabbage was the only sample that was found to be free of all the triazines analyzed. The order of contamination was observed to be propazine>atrazine>simazine>terbuthylazine. The concentrations obtained in all samples were below the MRL values which suggests that these fruits and vegetables from local middle markets do not pose threat to human health safety. However, the presence of these triazine residues in fruits and vegetables indicates the importance of their continuous monitoring for human safety consumption. Moreover, the results obtained in this work will contribute towards the generation of data on the presence of triazines in fruits and vegetables in the South African continent especially KwaZulu Natal.

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Chapter Six: Conclusions, Recommendations, and Research Shortcomings

6.1 Conclusion

The applicability of the proposed multi-residue analysis methods (QuEChERS, SPE, USE followed by LC-PDA) showed to be applicable for the determination of the selected triazines in fruits and vegetables. The optimization of the methods improved their extraction efficiency and precision which is crucial to obtain reliable residue data as well as ensuring the quality of marketed fruits and vegetables. The pre-treatment showed to be the effective way of removing pesticides from the surface, however, it does not eliminate the systematic pesticides present inside the fruit or vegetable. Simazine and propazine were found to be the most dominant triazines in fruit and vegetables. Carrot was the most polluted vegetable as it contained most of the analysed triazines, while cabbage was the only sample that was found to be free of all the triazines analysed.

In comparison of QuEChERS and SPE, both methods were found to be comparable in qualitative and quantitative determination of five selected triazines in fruits and vegetables. However, SPE showed to be slightly accurate while QuEChERS showed to be slightly sensitive. QuEChERS can therefore be recommended for routine analysis of these triazines due to its fewer extraction steps compared to SPE which is important for turn-around time. The USE method showed that USE with or without SPE clean-up is accurate for the determination of the selected triazines in the analyzed fruits and vegetables. However, USE without SPE clean-up showed to be more accurate and sensitive and hence can be recommended for effective daily monitoring of triazines in fruits and vegetables. Moreover, it is a cheap and easy, and environmentally friendly method. Furthermore, the LODs, LOQs obtained for all proposed methods were below the MRL values indicating the accurate applicability of the methods to extract trace levels of triazines in fruits and vegetables.

The concentrations obtained in all samples were below the MRL values which suggests that these fruits and vegetables from local markets do not currently pose threat to human health. However, the presence of these triazine residues in fruits and vegetables indicates the importance of their continuous monitoring for human safety upon consumption. Moreover, the results obtained in this work will contribute towards the lacking data on the presence of triazines in fruits and vegetables in the South African continent especially KwaZulu Natal.

They may also be used to inform the policy makers on the pollution levels and thus help them to apply remedial measures and create awareness.

6.2 Recommendations

- To expand the application of the optimized analysis methods to analyse the selected fruits and vegetables from various markets around South Africa to have a clear indication of the health risk the consumer from the South African continent is exposed to.

- To apply the optimized extraction techniques for monitoring of the selected triazines in other types of fruits and vegetables to asses if their presence is below the maximum residue limits.

- To develop methods for other classes of pesticides used in agricultural lands examine if their presence in food matrices is within the acceptable residue limits.

6.3 Research shortcomings

Storing of homogenized fruits and vegetable samples for more than three days inside or outside the refrigerator had a negative impact on the results. Since the analytes of interest tend to degrade or get lost with time, therefore it was difficult to analyze same samples during the cause of the experiment (method optimization, etc.). The research was also negatively impacted by the constant breaking down and maintenance of the LC-MS instrument which led to the research taking longer than two years. This problem added to the problem that was already there of the long waiting list of students that were using the LC-MS instrument for their research as well. Re-analysing similar samples if not satisfied with the results obtained in the previous run by the LC-MS instruments was also difficult since it was not possible to store samples for more than 3 days due to the loss of analytes from the fruits and vegetable samples as mention above.

Appendix

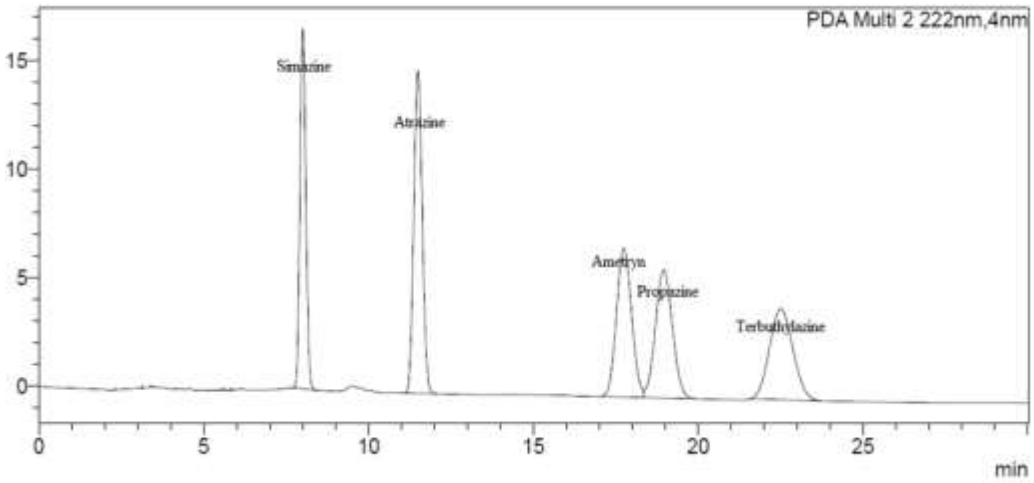

Figure S1: Simple chromatogram showing 5 five triazines (Simazine, Atrazine, Ametryn, Propazine, and Terbuthylazine) in a mixture/standard of 1ppm. Mobile phase composition (ACN:H_2O), LC-gradient was from 0-3min (42:58)v/v and 3-25min (30:70) v/v, wavelength, flow rates and injection volume were 222nm, 0.65mL/min, and 10 µL respectively.

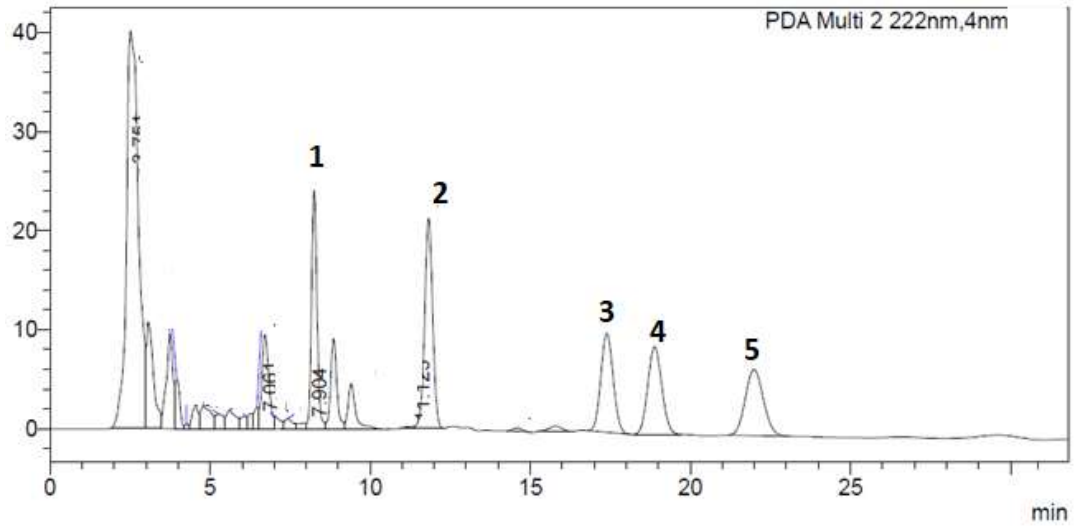

Figure S2: Chromatogram of potato sample fortified at 100 µg/kg

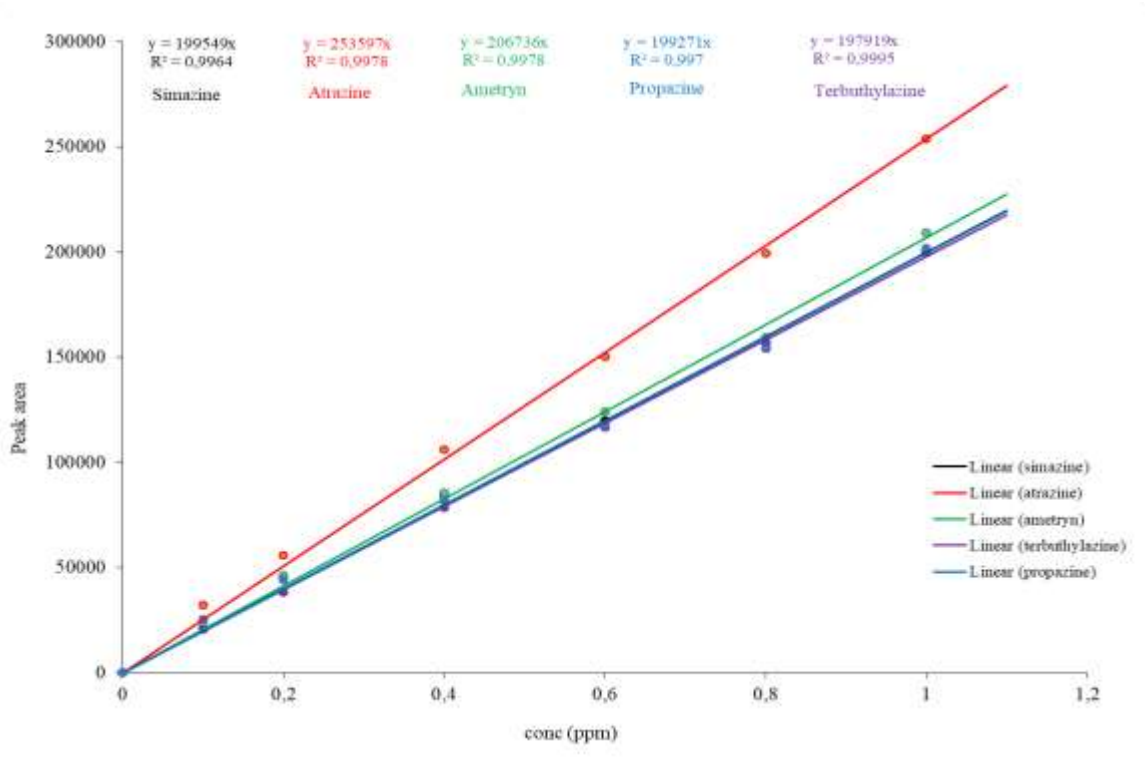

Figure S3: Calibration curves for triazine compounds obtained using LC-UV-PDA in Chapter 4.

Table S1: Significance of results on the effect of pH in QuEChERS method

t-Test: Two-Sample Assuming Unequal Variances		
	pH5	*pH7*
Mean	83.8	61.4
Variance	33.7	112.3
Observations	5	5
Hypothesized Mean Difference	0	
df	6	
t Stat	4.145306	
P(T<=t) one-tail	0.003021	
t Critical one-tail	1.94318	
P(T<=t) two-tail	0.006043	
t Critical two-tail	2.446912	

t-Test: Two-Sample Assuming Unequal Variances		
	pH5	*pH9*
Mean	83.8	55.8
Variance	33.7	107.7
Observations	5	5
Hypothesized Mean Difference	0	
df	6	
t Stat	5.265242	
P(T<=t) one-tail	0.000946	
t Critical one-tail	1.94318	
P(T<=t) two-tail	0.001892	
t Critical two-tail	2.446912	

t-Test: Two-Sample Assuming Unequal Variances		
	pH7	*pH9*
Mean	61.4	55.8
Variance	112.3	107.7
Observations	5	5
Hypothesized Mean Difference	0	
df	8	
t Stat	0.844232	
P(T<=t) one-tail	0.211533	
t Critical one-tail	1.859548	
P(T<=t) two-tail	0.423065	
t Critical two-tail	2.306004	

Table S2: Significance of results on the effect of extraction solvent in QuEChERS method

t-Test: Two-Sample Assuming Unequal Variances		
	ACN	*Acetic acid:ACN*
Mean	103.6	64.2
Variance	57.8	345.2
Observations	5	5
Hypothesized Mean Difference	0	
df	5	
t Stat	4.388627306	
P(T<=t) one-tail	0.003548498	
t Critical one-tail	2.015048373	
P(T<=t) two-tail	0.007096995	
t Critical two-tail	2.570581836	

t-Test: Two-Sample Assuming Unequal Variances		
	ACN	*ACN:MeOH*
Mean	103.6	62.8
Variance	57.8	1024.2
Observations	5	5
Hypothesized Mean Difference	0	
df	4	
t Stat	2.773521488	
P(T<=t) one-tail	0.025074918	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.050149837	
t Critical two-tail	2.776445105	

t-Test: Two-Sample Assuming Unequal Variances		
	Acetic acid:ACN	*ACN:MeOH*
Mean	64.2	65.2
Variance	345.2	1294.2
Observations	5	5
Hypothesized Mean Difference	0	
df	6	
t Stat	-0.055225866	
P(T<=t) one-tail	0.478875777	
t Critical one-tail	1.943180281	
P(T<=t) two-tail	0.957751555	
t Critical two-tail	2.446911851	

Table S3: Significance of results on the effect of pH in SPE method

t-Test: Two-Sample Assuming Unequal Variances

	pH5	*pH7*
Mean	72.2	105.2
Variance	87.7	69.2
Observations	5	5
Hypothesized Mean Difference	0	
df	8	
t Stat	-5.89098	
P(T<=t) one-tail	0.000183	
t Critical one-tail	1.859548	
P(T<=t) two-tail	0.000365	
t Critical two-tail	2.306004	

t-Test: Two-Sample Assuming Unequal Variances

	pH5	*pH9*
Mean	72.2	65
Variance	87.7	88.5
Observations	5	5
Hypothesized Mean Difference	0	
df	8	
t Stat	1.212871	
P(T<=t) one-tail	0.129888	
t Critical one-tail	1.859548	
P(T<=t) two-tail	0.259775	
t Critical two-tail	2.306004	

t-Test: Two-Sample Assuming Unequal Variances

	pH7	*pH9*
Mean	105.2	65
Variance	69.2	88.5
Observations	5	5
Hypothesized Mean Difference	0	
df	8	
t Stat	7.158058	
P(T<=t) one-tail	4.82E-05	
t Critical one-tail	1.859548	
P(T<=t) two-tail	9.63E-05	
t Critical two-tail	2.306004	

Table S4: Significance of results on the effect of sample volume in SPE method

t-Test: Two-Sample Assuming Unequal Variances

	25 Ml	*50 Ml*
Mean	91	83.6
Variance	14.5	228.8
Observations	5	5
Hypothesized Mean Difference	0	
df	5	
t Stat	1.06083	
P(T<=t) one-tail	0.168649	
t Critical one-tail	2.015048	
P(T<=t) two-tail	0.337298	
t Critical two-tail	2.570582	

t-Test: Two-Sample Assuming Unequal Variances

	25 ml	*100 ml*
Mean	91	107.8
Variance	14.5	28.7
Observations	5	5
Hypothesized Mean Difference	0	
df	7	
t Stat	-5.71548	
P(T<=t) one-tail	0.000362	
t Critical one-tail	1.894579	
P(T<=t) two-tail	0.000724	
t Critical two-tail	2.364624	

t-Test: Two-Sample Assuming Unequal Variances

	50 ml	*100 ml*
Mean	83.6	107.8
Variance	228.8	28.7
Observations	5	5
Hypothesized Mean Difference	0	
df	5	
t Stat	-3.37219	
P(T<=t) one-tail	0.009921	
t Critical one-tail	2.015048	
P(T<=t) two-tail	0.019842	
t Critical two-tail	2.570582	

Table S5: Significance of LODs and LOQs results for QuEChERS and SPE methods

t-Test: Two-Sample Assuming Unequal Variances		
	Q (LOD)	*SPE (LOD)*
Mean	0.84	1.28
Variance	0.163	0.137
Observations	5	5
Hypothesized Mean Difference	0	
df	8	
t Stat	-1.79629	
P(T<=t) one-tail	0.055088	
t Critical one-tail	1.859548	
P(T<=t) two-tail	0.110175	
t Critical two-tail	2.306004	

t-Test: Two-Sample Assuming Unequal Variances		
	Q (LOQ)	*SPE (LOQ)*
Mean	2.76	3.56
Variance	1.733	1.333
Observations	5	5
Hypothesized Mean Difference	0	
df	8	
t Stat	-1.02162	
P(T<=t) one-tail	0.168429	
t Critical one-tail	1.859548	
P(T<=t) two-tail	0.336858	
t Critical two-tail	2.306004	

Table S6: Significance of triazine recoveries and concentrations obtained using QuEChERS and SPE methods

t-Test: Two-Sample Assuming Unequal Variances		
	Q (Rec)	*SPE (Rec)*
Mean	95.2	106.8
Variance	46.7	25.2
Observations	5	5
Hypothesized Mean Difference	0	
df	7	
t Stat	-3.05899	
P(T<=t) one-tail	0.009175	
t Critical one-tail	1.894579	
P(T<=t) two-tail	0.018349	
t Critical two-tail	2.364624	

t-Test: Two-Sample Assuming Unequal Variances		
	Q conc	*SPE conc*
Mean	25.16667	30.16667
Variance	355.7667	820.9667
Observations	6	6
Hypothesized Mean Difference	0	
df	9	
t Stat	-0.35703	
P(T<=t) one-tail	0.364648	
t Critical one-tail	1.833113	
P(T<=t) two-tail	0.729296	
t Critical two-tail	2.262157	

Table S7: Correlation coefficient (R^2), LOD (µg/kg), LOQ (µg/kg), Recovery (%R) and RSD of the triazines in fruits and vegetables samples spiked at 100 µg/kg, (n=3) for SPE.

Triazines	**Apple**			**Pear**			**Banana**			**Orange**		
	LOD	LOQ	%R ± RSD	LOD	LOQ	%R ± RSD	LOD	LOQ	%R ± RSD	LOD	LOQ	%R ± RSD
Simazine	0.8	2.3	90±5	1.6	4.9	120±8	1.6	4.9	80±12	0.7	2.2	84 ±5
Atrazine	1.2	3.8	79±10	0.5	1.6	77±4	0.9	2.9	108±5	1.3	4.0	76±11
Ametryn	1.4	4.2	109±8	1.7	5.2	111±9	1.1	3.4	82±8	0.9	2.8	84±7
Propazine	0.8	2.6	104±5	1.0	3.1	104±6	1.2	3.7	91±8	0.9	2.7	95±6
Terbuthylazine	1.1	3.3	101±7	0.5	1.8	96±4	0.5	1.4	81±4	1.4	4.2	77±11
	Spinach			**Avocado**			**Potato**			**Cucumber**		
Simazine	0.5	1.4	101±36	1.0	3.2	84±8	1.8	4.9	111 ± 4	0.6	1.7	107±3
Atrazine	0.7	2.0	79±5	0.9	2.8	76±7	0.9	2.4	111 ± 6	0.5	1.6	100±3
Ametryn	1.2	3.7	97±8	0.3	0.9	70±3	1.0	2.8	109 ± 3	1.3	3.9	100±8
Propazine	0.7	2.0	89±4	0.1	3.8	98±8	1.2	3.0	103 ± 3	1.1	3.4	88±8
Terbuthylazine	0.7	2.3	102±4	0.1	4.1	119±7	1.5	4.7	100 ± 4	0.7	2.4	99±5

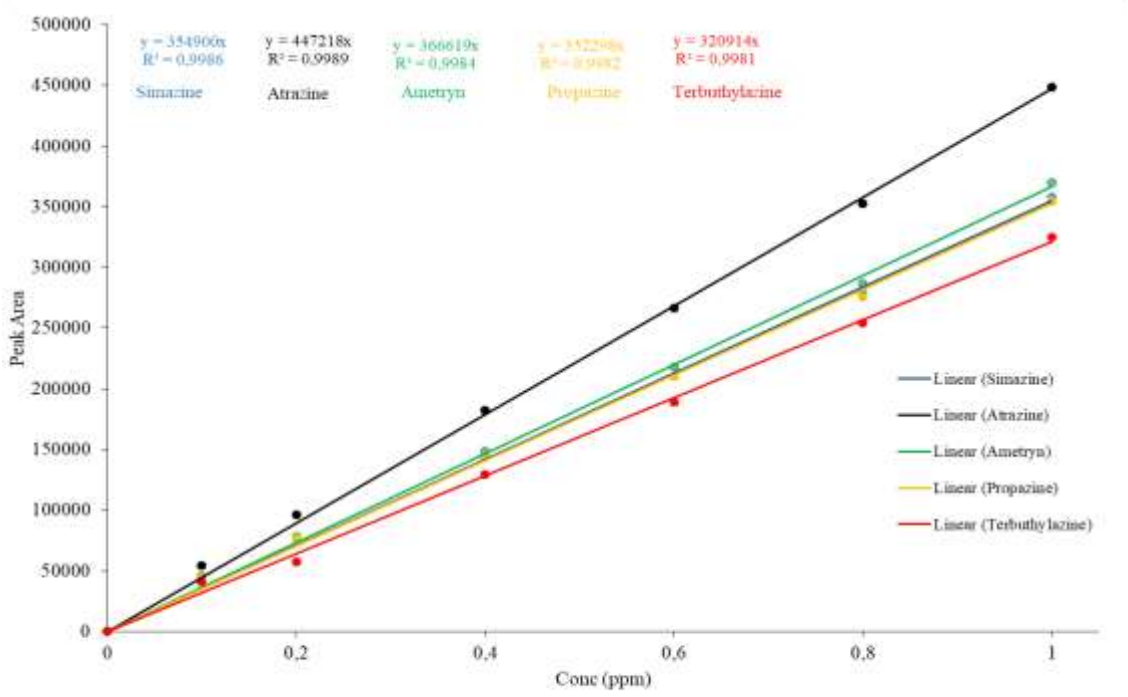

Figure S4: Calibration curves for triazine compounds obtained using LC-UV-PDA in Chapter 5.

Table S8: Significance of results on the influence of extraction solvent on the USE extraction efficiency without SPE clean-up

t-Test: Two-Sample Assuming Unequal Variances

	Acetone	*Acetonitrile*
Mean	48	101,6
Variance	1047,5	168,3
Observations	5	5
Hypothesized Mean	0	
df	5	
t Stat	-3,43731	
P(T<=t) one-tail	0,009244	
t Critical one-tail	2,015048	
P(T<=t) two-tail	0,018489	
t Critical two-tail	2,570582	

t-Test: Two-Sample Assuming Unequal Variances

	Acetone	*Ethyl acetate*
Mean	48	53,2
Variance	1047,5	511,7
Observations	5	5
Hypothesized Mean	0	
df	7	
t Stat	-0,2944675	
P(T<=t) one-tail	0,38847075	
t Critical one-tail	1,89457861	
P(T<=t) two-tail	0,7769415	
t Critical two-tail	2,36462425	

t-Test: Two-Sample Assuming Unequal Variances

	Acetone	*Methanol*
Mean	48	85,6
Variance	1047,5	419,3
Observations	5	5
Hypothesized Mean Difference	0	
df	7	
t Stat	-2,19527	
P(T<=t) one-tail	0,032089	
t Critical one-tail	1,894579	
P(T<=t) two-tail	0,064178	
t Critical two-tail	2,364624	

t-Test: Two-Sample Assuming Unequal Varianc

	Acetonitrile	*Ethyl acetate*
Mean	101,6	53,2
Variance	168,3	511,7
Observati	5	5
Hypothesi	0	
df	6	
t Stat	4,1502658	
P(T<=t) on	0,0030047	
t Critical o	1,9431803	
P(T<=t) tw	0,0060093	
t Critical tv	2,4469119	

t-Test: Two-Sample Assuming Unequal Variances

	Acetonitrile	*Methanol*
Mean	101,6	85,6
Variance	168,3	419,3
Observations	5	5
Hypothesized Mean	0	
df	7	
t Stat	1,475924	
P(T<=t) one-tail	0,091737	
t Critical one-tail	1,894579	
P(T<=t) two-tail	0,183474	
t Critical two-tail	2,364624	

t-Test: Two-Sample Assuming Unequal Variance

	Ethyl acetate	*Methanol*
Mean	53,2	85,6
Variance	511,7	419,3
Observations	5	5
Hypothesized Mean	0	
df	8	
t Stat	-2,374407	
P(T<=t) one-tail	0,02246812	
t Critical one-tail	1,85954804	
P(T<=t) two-tail	0,04493624	
t Critical two-tail	2,30600414	

Table S9: Significance of results on the influence of extraction solvent volume on the USE extraction efficiency without SPE clean-up

t-Test: Two-Sample Assuming Unequal Variances		
	20 mL	*30 mL*
Mean	77,2	101,6
Variance	10,7	168,3
Observations	5	5
Hypothesized Mean	0	
df	5	
t Stat	-4,0780102	
P(T<=t) one-tail	0,00477925	
t Critical one-tail	2,01504837	
P(T<=t) two-tail	0,0095585	
t Critical two-tail	2,57058184	

t-Test: Two-Sample Assuming Unequal Variances		
	20 mL	*40 mL*
Mean	77,2	70,6
Variance	10,7	211,3
Observations	5	5
Hypothesized Mean Difference	0	
df	4	
t Stat	0,990495	
P(T<=t) one-tail	0,189	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,378001	
t Critical two-tail	2,776445	

t-Test: Two-Sample Assuming Unequal Variance		
	30 mL	*40 mL*
Mean	101,6	70,6
Variance	168,3	211,3
Observatio	5	5
Hypothesi	0	
df	8	
t Stat	3,5578169	
P(T<=t) on	0,0037122	
t Critical o	1,859548	
P(T<=t) tw	0,0074243	
t Critical tv	2,3060041	

Table S10: Significance of results influence of extraction time on the USE extraction efficiency without SPE clean-up

t-Test: Two-Sample Assuming Unequal Variances		
	15 min	*30 min*
Mean	78,4	102
Variance	394,3	153,5
Observations	5	5
Hypothesized Mean Difference	0	
df	7	
t Stat	-2,25469	
P(T<=t) one-tail	0,029395	
t Critical one-tail	1,894579	
P(T<=t) two-tail	0,05879	
t Critical two-tail	2,364624	

t-Test: Two-Sample Assuming Unequal Variances		
	15 min	*45 min*
Mean	78,4	65,2
Variance	394,3	245,2
Observatio	5	5
Hypothesi	0	
df	8	
t Stat	1,1671822	
P(T<=t) on	0,1383731	
t Critical o	1,859548	
P(T<=t) tw	0,2767463	
t Critical tv	2,3060041	

t-Test: Two-Sample Assuming Unequal Variances		
	30 min	*45 min*
Mean	102	65,2
Variance	153,5	245,2
Observations	5	5
Hypothesized Mean	0	
df	8	
t Stat	4,121067	
P(T<=t) one-tail	0,00167	
t Critical one-tail	1,859548	
P(T<=t) two-tail	0,003339	
t Critical two-tail	2,306004	

Table S11: Significance of results on the influence of spiking with different concentrations on the USE extraction efficiency without SPE clean-up.

t-Test: Two-Sample Assuming Unequal Variances		
	0.05 ppm	*0.1 ppm*
Mean	100,6	99,6
Variance	94,3	166,3
Observations	5	5
Hypothesized Mean	0	
df	7	
t Stat	0,138515	
P(T<=t) one-tail	0,446867	
t Critical one-tail	1,894579	
P(T<=t) two-tail	0,893734	
t Critical two-tail	2,364624	

t-Test: Two-Sample Assuming Unequal Variances		
	0.05 ppm	*1.0 ppm*
Mean	100,6	104
Variance	94,3	93,5
Observations	5	5
Hypothesized Mean	0	
df	8	
t Stat	-0,554774	
P(T<=t) one-tail	0,29710792	
t Critical one-tail	1,85954804	
P(T<=t) two-tail	0,59421583	
t Critical two-tail	2,30600414	

t-Test: Two-Sample Assuming Unequal Variances		
	0.1 ppm	*1.0 ppm*
Mean	99,6	104
Variance	166,3	93,5
Observations	5	5
Hypothesized Mean Difference	0	
df	7	
t Stat	-0,61041	
P(T<=t) one-tail	0,28044	
t Critical one-tail	1,894579	
P(T<=t) two-tail	0,56088	
t Critical two-tail	2,364624	

Table S12a: Correlation coefficient (R^2), LOD (µg/kg), LOQ (µg/kg), Recovery (%R) and RSD of the triazines in fruits and vegetables samples spiked at 100 µg/kg, (n=3) for USE method without SPE clean-up for chapter 5.

Triazines	USE method without SPE clean up											
	Beetroot			**Bell Pepper**			**Cabbage**			**Grape**		
	LOD	LOQ	%R ± RSD	LOD	LOQ	%R ± RSD	LOD	LOQ	%R ± RSD	LOD	LOQ	%R ± RSD
Simazine	0.1	0.9	120 ±7.4	0.5	1.6	104 ± 1.5	0.5	0.9	84 ± 12.0	0.3	1.1	89 ± 9.1

Atrazine	0.5	1.9	115 ±11.5	0.3	1.5	107 ± 5.3	0.3	0.9	70 ± 7.0	0.5	1.6	101 ± 10.0
Ametryn	0.3	0.9	93 ±9.2	0.5	1.4	87.2 ± 4.4	0.2	1.3	106 ±5.3	0.4	1.4	104 ± 4.0
Propazine	0.4	1.3	98 ±9.8	0.9	1.6	113 ± 5.6	0.3	1.1	80 ±8.0	0.4	1.6	111 ± 9.8
Terbuthylazine	0.3	0.7	94±9.4	0.5	1.9	96 ± 4.8	0.2	1.0	64 ±6.4	0.3	1.3	85 ± 5.2
	USE method without SPE clean-up											
	Lemon			**Passionfruit**			**Peas**			**Plum**		
Simazine	0.2	1.5	102 ± 6.1	0.5	1.2	94 ± 16.0	0.7	1.8	88 ± 13.0	0.7	1.6	77 ± 6.7
Atrazine	0.3	1.9	102 ± 6.1	0.4	1.6	87 ± 7.0	0.7	1.0	74 ± 12.0	0.4	2.4	77 ± 7.0
Ametryn	0.2	1.1	108 ± 6.4	0.3	1.6	82 ± 9.0	0.3	1.8	93 ± 2.7	0.8	1.8	69 ± 7.6
Propazine	0.3	1.0	92 ± 7.6	0.3	1.1	93 ± 9.3	0.4	1.1	78 ± 6.4	0.4	1.5	86 ± 4.0
Terbuthylazine	1.0	1.8	116 ± 7.9	0.2	2.0	76 ± 5.9	0.3	1.9	98 ± 9.8	0.3	1.9	85 ± 5.2

Table S12b: Correlation coefficient (R^2), LOD (µg/kg), LOQ (µg/kg), Recovery (%R) and RSD of the triazines in fruits and vegetables samples spiked at 100 µg/kg, (n=3) for USE method with SPE clean up.

Triazines	USE method with SPE clean-up											
	Beetroot			**Bell Pepper**			**Cabbage**			**Grape**		
	LOD	LOQ	%R ± RSD	LOD	LOQ	%R ± RSD	LOD	LOQ	%R ± RSD	LOD	LOQ	%R ± RSD
Simazine	1.9	2.7	82 ± 14.2	1.9	3.5	74 ± 11.5	1.1	2.5	70 ± 9.3	1.3	2.0	78 ± 9.1
Atrazine	1.3	2.1	78 ± 10.0	1.5	2.9	77 ± 13.5	1.9	3.1	79 ± 11.0	1.1	2.8	90 ± 10.0
Ametryn	2.2	3.5	69 ± 12.1	1.1	2.0	82 ± 5.3	2.2	3.1	66 ± 3.8	2.4	3.3	74 ± 4.0
Propazine	2.0	4.0	65 ± 18.5	2.1	3.3	63 ± 8.3	1.3	2.5	70 ± 12.2	1.9	3.0	71 ± 9.8
Terbuthylazine	1.5	2.7	72 ± 14.1	1.8	1.9	69 ± 16.2	2.5	3.4	66 ± 5.5	1.6	3.5	70 ± 5.2
	USE method with SPE clean-up											
	Lemon			**Passionfruit**			**Peas**			**Plum**		
Simazine	2.5	3.9	62 ± 5.5	1.6	3.0	70 ± 11.9	1.2	2.9	80 ± 3.6	1.0	2.2	71 ± 8.9
Atrazine	1.5	2.9	80 ± 11.1	2.0	3.2	86 ± 2.3	1.5	3.1	71 ± 9.0	1.7	3.3	75 ± 6.2
Ametryn	1.2	2.1	74 ± 9.6	2.2	3.2	77 ± 1.8	0.9	2.3	90 ± 10.7	1.2	3.0	68 ± 11.0

Propazine	2.0	3.0	91 ± 10.6	1.5	2.7	70 ± 10.3	2.5	3.3	71 ± 5.5	2.3	3.3	69 ± 15.0
Terbuthylazine	1.8	2.6	71 ± 8.5	1.8	2.9	81 ± 6.6	2.0	2.9	85 ± 14.2	2.3	3.0	78 ± 12.6