ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF CHEMISTRY

Analytical Sample Preparation Methods Based on Miniaturized Extraction for Trace Level Enrichment of Selected Pesticide Residues from Environmental Waters and Food Samples Utilizing High Performance Liquid Chromatographic Analysis

By

Habtamu Bekele

Supervisors:

Prof. Negussie Megersa

Dr. Weldgebriel Yohannes

November, 2023

Addis Ababa, Ethiopia

Analytical Sample Preparation Methods Based on Miniaturized Extraction for Trace Level Enrichment of Selected Pesticide Residues from Environmental Water and Food Samples Utilizing High Performance Liquid Chromatographic Analysis

By

Habtamu Bekele

A Thesis Submitted to the Department of Chemistry, College of Natural and Computational Sciences, Addis Ababa University in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Chemistry (Analytical Chemistry)

November, 2023

Addis Ababa, Ethiopia

APPROVAL SHEET

Addis Ababa University

College of Natural and Computational Sciences

Department of Chemistry

This is to certify that the thesis prepared by Habtamu Bekele entitled "Analytical Sample Preparation Methods Based on Miniaturized Extraction for Trace Level Enrichment of Selected Pesticide Residues from Environmental Water and Food Samples Utilizing High Performance Liquid Chromatographic Analysis", submitted in partial fulfillment of the requirements of the degree of Doctor of Philosophy (Analytical Chemistry) complies with the regulation of the University and meets the standards with respect to quality and originality.

Approved by the examining committee:

Name	Signature	Date
Prof. Abera Gure (External Examiner)		Dec. 11, 2023
Prof. B. S.Chandravanshi (Internal Examiner)		Dec. 11, 2023
Dr. Ahmed Hussen (Internal Examiner)		Dec. 11, 2023
Prof. Negussie Megersa (Advisor)		De. 11, 2023
Dr. Weldgebriel Yohannes (Advisor)		Dec. 11, 2023
Prof. Ahmed Mustefa (Chairman, Department of Chemistry)		Dec. 13/2023

DEDICATION

To

My mother Boge Moti, my beloved wife, Ayane Fixuma, my children: Simera, Sifan, Nimoonaa, and Maraaol, and my late father Bekele Sime.

STATEMENT OF AUTHOR

I, hereby, declare that this thesis submitted for the degree of Doctor of Philosophy in Chemistry (Analytical Chemistry), at Addis Ababa University, College of Natural and Computational Sciences, Addis Ababa, Ethiopia, is my own original work and has not been submitted previously to any institution. All sources of materials used in this work have been duly acknowledged.

Habtamu Bekele

November, 2023

TABLE OF CONTENTS

LIST OF TABLES

Tables

LIST OF FIGURES

Figures

LIST OF ABBREVIATIONS

Abbreviation	Description
CNTs	Carbon Nano Tubes
DDT	Dichlorodiphenyltrichloroethane
DLLME	Dispersive Liquid Liquid microextraction
DLLME-SFOD	DLLME based on Surface Floating Organic Droplets
DSPE	Dispersive Solid Phase Extraction
EF	Enrichment Factor
EU	European Union
G	Graphene
GC	Gas Chromatography
GO	Graphene Oxide
HD-DLLME	High Density Dispersive Liquid Liquid Microexxtraction
HF-LPME	Hollow Fiber Liquid Phase Microextraction
HLLME	Homogeneous-Liquid Liquid Microextraction
HPLC-DAD	High Performance Liquid Chromatographic coupled with Diode Array Detector
IT-SPME	In-Tube Solid Phase Microextraction
LC–MS	Liquid Chromatography coupled Mass Spectrometry
LC-MS/MS	Liquid Chromatography coupled with Tandem Mass Spectrometry
LLE	Liquid Liquid Extraction
MRLs	Maximum Residue Levels
OCPs	Organochlorine Pesticides
OPPs	Organophosphorus Pesticides
2,4-D	Dichlorophenoxyacetic acid
4,4'-DDD	4,4'-Dichlorodiphenyldichloroethane
4,4'-DDE	4,4'-Dichlorodiphenyldichloroethylene
4,4'-DDT	4,4'-Dichlorodiphenyltrichloroethane
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
R	Extraction Recovery

RPLC	Reversed-Phase Liquid Chromatography
RR	Relative Recovery
SA-GO-DSPE	Salt Assisted Graphene Oxide sorbent Dispersive Solid Phase
SALLE	Salting-out Assisted Liquid-Liquid Extraction
SDME	Single Drop Microextraction
SPE	Solid Phase Extraction
SPME	Solid Phase Microextraction
ST- DLLME	Solvent Terminated Dispersive Liquid–Liquid Microextraction
SUHs	Sulfonylurea Herbicides
UV	Ultra Violet
WHO	World Health Organization

ACKNOWLEDGEMENTS

I want to start by expressing the most profound gratitude to the Almighty God. I present Him all the glory and honor for leading me on this journey and giving me the strength and knowledge through the Holy Spirit.

I would like to express my sincere gratitude to my supervisor Prof. Negussie Megersa for accepting me in his separation science research group and providing consistent supervision throughout completion of my PhD study. I want to thank him for his day to day advice without any limitation of time and space. I also thank him for his insightful comments, corrections and suggestions during the courses, seminars, manuscript preparations and thesis write-up. Without his support and guidance, I would not be able to reach this level of work. Especially, I am thankful to Prof. Negussie for opening the opportunities to participate and present one part of our research work on international conference held in Arusha, Tanzania. He has taught me not only how to become a better separation science researcher but exhibited qualities I will carry with me for the rest of my life that are important to be an effective supervisor.

My heartfelt special thanks and appreciation goes to my co-supervisor Dr. Weldegbriel Yohannes for his encouragement, valuable advice and constructive comments during the courses, seminars and the research work. I greatly value all of his contributions and suggestions.

My special thanks go to Dr. Merid Tessema, Dr. Negussie Negash and Prof. B. S. Chandaravanshi for their constructive comments and positive evaluation during seminar presentation. My special thanks also go to Prof. Yonas Chebude for his assistance with the FTIR analysis and Dr. Estifanos Ele for his technical assistance when I faced problems with the HPLC instrument.

I acknowledge Dr. Teshome Tolcha for familiarizing me with HPLC-DAD and GC-MS instruments while he was too busy to do so. My gratitude also goes to my colleagues Abi leggese and Tura Gemechu who are members of our research group for their valuable discussions, cooperation and motivation in every aspect of this thesis work. I am also very grateful to my friends Dr. Gezaw Tesfaye, Tujuba Beyene, Bekele Habte, Miniyashal Alemayehu, Wondimeneh Dubale,

Mesfin Mokonnen and Shallama Guzo for their motivation, constructive ideas and their special support during my PhD study.

I want to express my sincere gratitude to Prof. Ahmed Mustefa, the current Head of the Department of Chemistry, Dr. Negash Getachew, the former Head of the Department of Chemistry and Dr. Mokonnen Abebayehu for their continuous follow up progress of research work during my study and I want to thank the academic and technical staff at the Department of Chemistry, Addis Ababa University for their timely assistance in this research work. I would like to acknowledge Oromiya Education Bureau, Oromiya, Ethiopia, for sponsoring me to pursue my PhD study. I am thankful to Mr. Solomon H/Mariyam, former dean of Fitche College of Teachers Education for facilitating all the administrative issues throughout my study leaves.

My most sincere gratefulness goes to my mother Boge Moti and my father Bekele Sime who have disciplined and constant prayers to arrive at this level. Especially, had it not been for the continuous follow ups, guidance and encouragements of my late father, I would have not arrived at this stage. My sisters Fayise and Tegibar as well as my brothers Ketema, Reta, Ashabir and Mitiku are all acknowledged for their unconditional love, inspiration, encouragement and support throughout my journey. My special thanks should go to my father-in-law Mr. Fixuma Berhanu and his family for their enduring motivation, moral support and praying for me and my family for all aspects of our life.

Last but not least, I would like to express my sincere gratitude to my lovely wife, Ayane Fixuma, who has supported me in my studies for the past six years by taking on all of our family responsibility. Your dedication, encouragement, and understanding during the challenges of financial, many ups and downs we encountered are deeply appreciated and will always be in my thoughts and memories. My children Simera, Sifan, Nimoonaa and Maraaol are also highly acknowledged and appreciated for their understanding, patience, and love throughout my study period.

Abstract

The development and implementation of more environmentally friendly and sustainable microextraction techniques in recent years has provided alternative to traditional sample preparation approaches. In this thesis, we developed, optimized and validated different micro extraction methods for trace level multi residue and multiclass trace level pesticides from environmental water and food samples prior to their determination using chromatographic techniques. Salt assisted graphene oxide (GO) was utilized as a sorbent in dispersive solid phase extraction (DSPE), to enhance and extract certain *s*-triazine herbicides from environmental water samples. GO can generate scattered colloids in the aqueous phase and offers an extensive variety of functional groups for hydrogen bonding or electrostatic interaction with benzene ring organic compounds with functional groups comprising oxygen and nitrogen. GO was synthesized by modified Hummers method and characterized with Fourier transform infrared (FT-IR) spectroscopy. As complete collection of GO after adsorption process is difficult task, in this established salt-assisted (SA)-GO-DSPE method, it was found to be aggregated and centrifuged in the presence of NaCl. The optimum value for main parameters that influenced extraction efficiency were investigated. Under the optimized conditions, low limits of detection (LODs) (0.12–0.80 ng mL^{-1}), limits of quantification (LOQ) (0.4–2.68 ng mL^{-1}), and good extraction recoveries (69.06–95.53%) were obtained for simultaneous extraction of simazine, atrazine, propazine and prometryn. The relative standard deviations (RSD) of intra-day (n = 6) and inter-day (n = 9) precisions were 2.54 to 5.84% and 4.38 to 8.56%, respectively. When the method's applicability was ultimately assessed, the target analytes in the real water samples weren't detected. The proposed method is environment friendly and promising for trace analysis of other pesticides including the target herbicides in environmental water samples.

Dispersive liquid–liquid microextraction based on surface floating organic droplet was designed to enable development of simple, fast, and environmentally friendly analytical technique utilizing for selective and quantitative enrichment of trace level pesticide contaminants from different fruit juice samples for subsequent detection by high pressure liquid chromatography–diode array detection (HPLC–DAD). The optimized method was investigated using spiked blank sample, and results of linearity at concentration levels from 3 to 1500 ng mL^{-1}, with correlation coefficients (r^2) ≥ 0.998 were obtained. LOD and LOQ were ranged from 1.3×10^{-2} to 5.3×10^{-2} μg L^{-1} and

4.2×10^{-2} to 1.8×10^{-1} μg L^{-1}, respectively. Satisfactory accuracy, *i.e,* recoveries ranging from 87.23 to 99.45%, with %RSD between 1.37 and 8.39, and precision in terms of %RSD ≤ 10.78 were investigated. At the end, the method was successfully applied to analyze real fruit juice samples and target analytes were not detected in real samples.

A simple, selective and fast salting out assisted liquid–liquid extraction (SALLE) technique coupled with HPLC–DAD was developed and optimized for extraction, and analysis of seven multiclass pesticide residues in pasteurized and raw cow milk samples. Under optimum conditions, for broad linear range concentration of 2–1500 μg L^{-1}, r^2 was within a range of 0.9982–0.9997. Reliable sensitivity was achieved with LODs and LOQs ranging from 0.58–2.56 ng mL^{-1} and 1.95–8.51 ng mL^{-1}, respectively. While precision with intra-day and inter-day in terms of RSDs were observed in the range of 1.97–7.88% and 4.52–8.13%, respectively. The results of the precision studies reveal that good repeatability and reproducibility (RSDs < 9%) were achieved. Finally, the validated approach was effectively used to extract and determine pesticide residues in real milk matrices, however the target analytes were not detected in all samples.

High density dispersive liquid–liquid microextraction method coupled HPLC-DAD was developed for extraction, enrichment and determination of six sulfonylurea herbicides; namely, metsulfuron-methyl, chlorosulfuron, niclosulfuron, prosulfuron, flazasulfuron and chlorimuron-ethyl in matrices of environmental waters. The optimum experimental parameters that influenced extraction efficiency were investigated. Under the optimized conditions, wide linear calibration curves with $r^2 > 0.997$, LODs in the range of 0.8–1.5 ng mL^{-1}, and LOQ in the range of 1.9–5.1 ng mL^{-1} were obtained. The precisions in terms of % RSDs of intra-day (n = 6) and inter-day (n = 9) were found to be 3.01 to 8.36 and 2.92 to 9.78, respectively. Applicability of the developed method was investigated by analyzing spiked tap, lake, river and underground water samples and satisfactory recoveries were obtained in the range of 84.3–101.7% with RSDs < 9.8% (n=6) and the target analytes were not detected in real samples.

Keywords: Azoxystrobin, Cow milk, Dispersive liquid–liquid microextraction, Environmental water, Fruit, HPLC, Salt assisted graphene oxide, Sulfonylurea herbicides, Triazine herbicide

1. INTRODUCTION

The substantial usage of a wide range of harmful chemicals is mainly linked to industrial and agricultural operations. In the production of agricultural products, farmers are regularly using pesticides to control weeds and insects, and it has been noted that these have led to notable gains in agricultural output. Without an equal increase in food production, the world's population growth in the 20th century would not have been possible. The use of pesticides affects the production of about one-third of agricultural outputs. Fruit, vegetable, and cereal output would all decrease by 78%, 54%, and 32%, respectively, without the use of pesticides. In order to decrease diseases and increase crop yields globally, use of pesticides are essential (Tudi et al., 2021). Through the obvious release into the environment, chemical wastes produced by industrial processes were disposed of. While liquids were efficiently diluted into receiving waters and carried away from the source, gases promptly diffused into the atmosphere. Likewise, pesticides that revolutionized agriculture and forest productivity are adversely affect non-target organisms and ecosystem as a whole (Naureen et al., 2020).

Pesticides are compounds that are either chemical substances or biological agent, such as bacteria or viruses, and are used to suppress, eradicate, or repel pests in order to boost agricultural outputs. Typically, the manufacturer of pesticides formulates the active ingredient as solid particles or emulsifiable concentrates. To utilize many commercial preparations, they are diluted with water first (Hazra, 2019). Some of the pesticides are persistent and some degrade in the environment giving various degrees of toxicity and distribution in similar manner as their parent compounds.

According to Antônio et al. (2021); Ramos et al. (2020); and Peris et al. (2022), the application of these compounds and the products of their degradation pose the risk of contaminating ground and surface waters, soil, and sediments. This could be dangerous for the environment and human health. Additionally, the compounds high water solubility may enable them to be carried to environmental water systems including spring, underground, and river waters, where they might eventually enter the food chain and be hazardous to soils, aquatic life, and people (Peris et al., 2022). This potential impact on the public health is more sever in developing countries, like Ethiopia, where pesticides are used by untrained farmers (Tessema & Nagy, 2021).

The majority of Ethiopians engage in agriculture and use pesticides to protect food and realizing higher crop yield by controlling pests. Many people use pesticides for lawn and garden uses, as well as inside and outside the homes, while others use them professionally for public health initiatives and commercial applications. However, pesticides have been identified as environmental contaminants when moved outside the application geographical areas, and as a result, are subject to control and monitoring (Teklu et al., 2016).

Farmers in developing countries frequently utilize pesticides arbitrarily and without the awareness or expertise to do so. They also utilize long-lasting, and highly hazardous insecticides. Negative effects on human health and the environment have been caused by improper management, a lack of skilled operator, and a lack of pesticide disposal facilities (Tessema & Nagy, 2021). Pesticides are improperly used and imported into Ethiopia without adequate controls, which inevitably has negative consequences on ecosystems and agricultural workers health. The lack of understanding among users of pesticides regarding their toxicological and chemical characteristics complicates the adverse health effects of these chemicals in Ethiopia (Negatu et al., 2016).

Some persistent pesticides, such as *s*-triazine and dichlorodiphenyltrichloroethane (DDT), have been banned in developed countries because they are persistent and have toxic effects on the environment and human health (Ago et al., 2023; Mengistie et al., 2015). Despite the fact that pesticides protect crops from pests and increase crop productivity, many pesticides have been banned from use and even production. According to the studies by Gebremichael et al. (2013) and Oesterlund et al. (2019), they are still used for medical and agricultural uses in developing countries like Ethiopia. The presence of contaminating pesticide residues in samples collected from various places in some localities of Ethiopia was determined by a number of studies that looked at these pesticides in various matrices. The results revealed the presence of ametryne in irrigation water (Tolcha & Megersa, 2018), 4,4'-DDT and its metabolites in Khat stables (Mekonen et al., 2017), 4,4'-DDT and its metabolites in commonly consumed agricultural crops like teff, maize, and red pepper (Mekonen et al., 2014), 4,4- DDE and 4,4-DDD in human and cow milk (Gebremichael et al., 2013) and γ-chlordane, endrin, and endosulfan sulfate in processed different brands of tea (Siraj, 2021). The analysis of these pesticides requires selective and effective sample preparation techniques that can simultaneously extract and preconcentrate them due to their

presence at trace levels and the complexity of environmental and food samples as this issue is the world frontier topic in the field of chemical separation science (Boulanouar et al., 2018).

It is well recognized and documented that majority of pesticides released into the environment are hazardous. Consequently, the World Health Organization (WHO), the European Union (EU), and the Food and Agriculture Organization of the United Nations (FAO) have enforced regulations on the maximum residue limits (MRLs) of these compounds in water and food matrices in small quantities, usually in the range of a few ng/L to μg/L, in order to protect consumer safety (Hamilton et al. 2003; WHO 2017). For instance, the Council of the European Communities (EU) has mandated that the concentration of pesticide residues in environmental waters meant for human consumption cannot be higher than 0.1 μg/L for individual pesticides and 0.5 μg/L for total pesticide concentrations (Council of the European Communities 1998, Council Directive 98/83/EC). Similarly, EU (2005, Regulation (EC) No. 396/2005) has set maximum residue levels (MRLs) for these compounds in a variety of food categories, such as raw fruits and vegetables, which can range from 0.01 to 5 mg/kg, depending on the specific compound. Additionally, 0.01 mg/kg is the default value for matrices such as fruit juices that are not covered by the regulation (Xu et al., 2020). As a result, efficient, quick, affordable, environmentally safe, sensitive, and selective multiresidue techniques are required.

Solid phase extraction (SPE) and liquid–liquid extraction (LLE) are frequently used as the sample pretreatment techniques for analyzing pesticide residues at trace levels (Timofeeva et al., 2017; Wang et al., 2019a; Zhao et al., 2019). Although LLE can provide excellent reproducibility and high sample capacity, it has drawbacks including a difficulty in automation, a lengthy operation, the use of a large volume of sample, and the use of high volume of harmful organic solvent. SPE can overcome some disadvantages of LLE such as high consumption of both sample and organic solvent, but it is also time consuming steps such as column conditioning, washing, loading and elution. On the other hand, sorbent ready to use in SPE is relatively expensive, sample carry over effect and also associated with the problem to choice the appropriate sorbent (Han et al., 2021; Liang et al., 2019; Samsidar & Shaarani, 2018).

The development of more environmentally friendly extraction solvents and miniaturizing extraction techniques have recently received a lot of interest since they have the potential to significantly reduce the aforementioned drawbacks. Dispersive liquid–liquid microextraction (DLLME) technique which is the miniaturized format of traditional LLE and dispersive solid phase extraction (DSPE) based carbon nanomaterials including graphene sorbent is more recent mode of conventional SPE techniques are intensively exploited to extract organic pollutants including pesticide residues from environmental water and food samples (Beshana et al., 2022; Bhattacharyya et al., 2023; Li et al., 2021; Liu et al., 2022; Li et al., 2015).

1.1. Research gap

Despite of substantial technological advances in analytical instruments, sample preparation to clean up the matrices, separate and/or concentrate the analytes of interest while rendering them into the form that is compatible with analytical systems is unavoidable in analysis (Farajzadeh et al., 2021). The development of sensitive, selective and reproducible analytical methods have always been a prerequisite for the achievement of high quality results in enforcement and monitoring programs. Conventional sample preparation techniques such as solid-liquid extraction methods like Soxhlet extraction (Kunene & Mahlambi, 2020); LLE and SPE (Timofeeva et al., 2017; Wang et al., 2019a; Zhao et al., 2019) are still widely in use for extraction of trace pesticide residues in environmental and food matrices. However, these methods are expensive, labor intensive, time consuming and require the use of large volumes of toxic organic solvents (Bhattacharyya et al., 2023).

Researchers have recently placed a high priority on advancing the development of efficient, inexpensive, automated, miniaturized, and environmental friendly extraction procedures that significantly minimize the usage of organic solvents. Therefore, in trace analysis, green extraction and/or miniaturized sample preparation techniques are interesting (Bernard i et al., 2022; Bhattacharyya et al., 2023; Farajzadeh et al., 2023). To this end, single drop microextraction (SDME) (Salemi et al., 2013; Viñas et al., 2010), hollow fiber liquid phase microextraction (HF-LPME) (Gure et al., 2013; Megersa, 2015), different modes of DLLME (Beshana et al., 2022; Carbonell-rozas et al., 2020; Farajzadeh et al., 2021; Li et al., 2021; Ramos et al., 2020; Tian et al., 2017; Tursen et al., 2021; Wang et al., 2016a), solid phase microextraction (SPME) (Chen et

al., 2019; Yang, 2017), easy, cheap, effective, rugged and safe extraction (QuEChERS) (Rejczak & Tuzimski, 2017), SALLE (Akram et al., 2017; Alemayehu et al., 2017; Negussie et al., 2021; Rouhollah & Rezvan, 2021; Tolcha & Megersa, 2021) and DSPE (Gao et al., 2019; Mahpishanian & Sereshti, 2014; Mohammadnia et al., 2020; Shi et al., 2017; Song et al., 2014; Sun et al., 2018; Zhang et al., 2017) techniques are significantly reducing or avoiding the use of organic solvents in sample preparation procedures have been developed and are now widely used.

SPE has been extensively explored in various formats including stir bar sorptive extraction (Sanchez-Ortega et al., 2009), solid phase membrane tip extraction which involving the use of tiny cone shaped membrane tip protected multiwall carbon nanotubes (See et al., 2010), DSPE (Chen et al., 2015a), SPE using hydrophilic lipophilic balanced sorbent (Ntombela & Mahlambi, 2019), micro SPE using titanium dioxide (TiO_2) nanotube array as sorbent (Zhou & Fang, 2015b) and sol-gel poly (ethylene glycol coated stir fabric phase sportive extraction (Roldán-Pijuán et al., 2015) and so forth were published methods for extraction of triazines in environmental water samples. Sorbent used in some reported methods are commercially prepared, expensive and not available in routine laboratories. Preparation of sorbents are also time-consuming procedure, require many organic and inorganic compounds which are not cost effective and ecofriendly. Graphene based materials have been used as adsorbents for the extraction and preconcentration of pesticides such as carbamate (Gao et al., 2019; Song et al., 2014), organophosphorus (Mahpishanian & Sereshti, 2016; Sun et al., 2018), organochlorine (Mohammadnia et al., 2020), triazine (Zhang et al., 2017; Zhao et al., 2011) and neonicotinoid (Shi et al., 2017). Most of the cited literatures and others use magnetized graphene based nanomaterials and functional composite of GO. Thus, synthesis of GO nanoparticle sorbent which possess high adsorption capacity, and low organic solvent consumption (in agreement with the principles of Green Chemistry) was important sorbent for extraction and preconcentration of *s*-triazine pesticides in environmental water samples.

Different modes of DLLME for preconcentration of multiple pesticide residues in environmental water samples and variety of food matrices such as vegetables and fruit juices have become widely used in recent years as a result of the replacement of traditional sample preparation techniques with DLLME (Heidari et al., 2020; Wang et al., 2018a; Wang et al., 2017; Wang et al., 2016b).

Although conventional DLLME, which commonly uses chlorinated solvents, has many advantages including ease of use, speed, affordability, high recoveries, and a high enrichment factor (Rai et al., 2016), the widespread use of halogenated solvents as extraction solvents has been constrained by their toxicity, which is also linked to human cancers. As a result, usage of chlorinated solvents has been outlawed globally and gradually been replaced with more environmentally friendly solvents that are lighter in density than water to address these shortcomings (Farajzadeh, 2019; Jouyban et al., 2020). After its development, numerous research works have been reported for analysis of pesticides in foods including fruit juices (Ahmadi-jouibari et al., 2017; Farajzadeh, 2019; Jouyban et al., 2020; Majlesi & Massoudinejad, 2016; Mao, 2020; Pirsaheb et al., 2015; Pirsaheb et al., 2017; Sharafi et al., 2015). But low density extraction solvent based DLLME for simultaneous extraction of multiclass pesticides in fruit juices were scarce in reported literatures. Thus, DLLME based on surface floating organic droplets (DLLME-SFOD) which is found to use less solvent volume, shorter extraction time, and eco-friendly was developed for selective extraction and enriching of multiclass pesticides from a variety of complex matrix fruit juice sample.

Among numerous extraction techniques that have been performed to achieve efficient extraction of pesticides from milk and milk products, LLE (Chen et al., 2014), SPE (Wang et al., 2019a), DSPE (Zhao, et al., 2016), magnetic solid phase extraction (MSPE) (Campos do Lago et al., 2020; Nodeh et al., 2016), SPME (Chen et al., 2019; Rodrigues et al., 2011; Yang, 2017), QuEChERS (Rejczak & Tuzimski, 2017), pressurized liquid extraction (Mezcua et al., 2007), and cloud point extraction (Mohd et al., 2018; Niu et al., 2017) were reported on some previous literature works. The majority of these methods are labor intensive, time consuming, expensive and environmentally unfriendly. In addition, as stated explicitly in published literatures, industrially produced QuEChERS kits, SPME needles, and SPE cartilage materials are quite expensive (Lachat & Glauser, 2018; Nováková & Vlčková, 2009; Samsidar & Shaarani, 2018). Therefore, there is a demand of solvent based microextraction techniques which is simple, cost effective and ecofriendly for enrichment and extraction of the trace level pesticide residues in milk samples prior to instrumental analysis. In this thesis, the proposed SALLE method for extraction of multiclass pestcides from cow milk samples is simple and cost effective, unlike the SPE method, it does not require multi-steps conditioning, washing, loading and elution (Lachat & Glauser, 2018; Samsidar

& Shaarani, 2018). In addition, unlike QuEChERs, the SALLE method integrate cleanup and preconcentration in a single step.

The development of efficient, inexpensive, automated, and miniaturized extraction techniques that could significantly reduce consumption of toxic organic solvents has recently been the focus and commitment of a large group of research endeavors (Jain et al., 2021; Liu et al., 2022). To this end, SDME (Salemi et al., 2013; Viñas et al., 2010), HF-LPME (Gure et al., 2013; Megersa, 2015) and SPME (Chen et al., 2019; Yang, 2017) techniques significantly minimized and working towards avoiding the use of organic solvents in sample preparation procedures. However, SPME is expensive, fiber is fragile, and has a short lifetime and takes a long time to condition the sorbent (Chen et al., 2019). Despite its ease of use and effectiveness, SDME is merely used for laboratory research due to its drop instability and the main drawbacks of HF-LPME are poor reproducibility and lengthy equilibration times (Samsidar & Shaarani, 2018). DLLME is a modified version of solvent extraction which has the ability of enhancing enrichment intensely, and contains very small amount of toxic solvent used and is the technique in which acceptor to donor phase ratio is greatly reduced compared to other methods used for similar purposes (Liu et al., 2022; Wang et al., 2016b; Wu et al., 2010). A microextraction technique, DLLME provides features such as ease of use, fast, high recovery, and the use of inexpensive widely accessible laboratory supplies (Huang et al., 2020). The technique has commonly been used to extract trace levels of pesticides ever since it was first introduced, mostly from water samples (Beshana et al., 2022; Cheng et al., 2010; Ramos et al., 2020; Wang et al., 2016a), foods (Carbonell-rozas et al., 2020; Li et al., 2021; Tian et al., 2017), and especially from juices and vegetables (Farajzadeh et al., 2021; Tursen et al., 2021). However, there are very few literature reports on application of the technique for determination of sulfonylurea herbicides (SUHs) in waters and none on the use of high density solvent DLLME (HD-DLLME), for simultaneous extraction of SUH residues, e.g., metsulfuron-methyl, chlorosulfuron, niclosulfuron, prosulfuron, flazasulfuron and chlorimuron-ethyl in the matrices of environmental waters (tap, underground, lake, and river) prior to HPLC-DAD analysis.

Therefore, the purpose of the current study consisted of filling the aforementioned research gaps. Accordingly, certain suitable miniaturized and/or green sample preparation techniques for

the selective preconcentration, extraction, and quantitative detection of trace amounts of chosen pesticide residues in various environmental water and food sample matrices were established.

1.2. Objectives of the research

1.2.1. General objective

The general objective of the research is to develop different miniature analytical sample preparation methods for extraction and analyzing trace amounts of pesticide residues in water and food matrices.

1.2.2. Specific objectives

The specific objectives of the research works are:

a) To develop a miniaturized sample preparation technique based on SA-GO-HPLC-DAD for selective preconcentration, extraction, and quantitative determination of trace level selected *s*- triazine pesticide residues in different environmental water samples.
b) To develop and optimize DLLME-SFOD-HPLC-DAD for trace analysis of multiclass pesticide residues in different fruit juice samples.
c) To develop SALLE method coupled with HPLC-DAD for trace enrichment of multiclass selected pesticide residues in cow milk.
d) To develop and optimize important parameters of HD-DLLME coupled with HPLC-DAD for analysis of relatively polar selected sulfonylurea pesticide residues in environmental water samples.

1.3. Significance of the study

This thesis work aimed to developed modern sample preparation procedures like SA-GO-DSPE, DLLME, and SALLE followed by the chromatographic technique, HPLC–DAD for chromatographic determination, and to address the knowledge gap that currently exists in the country for selected pesticide analysis in some environmental waters and food samples. The developed techniques were tested and used to analyze a few specific types of pesticides that have been legally used in Ethiopia for a long time such as (*s*-triazine, sulfonylurea, and phenylurea) herbicides, (carbamate, organophosphorus, and neonicotinoid) insecticides, as well as fungicide in environmental water and food samples. As a result, the thesis work offered the scientific report on the analytical procedures

developed for trace level extraction and analysis of some chosen classes of pesticides researched in the country. Thus, the findings of the current study will primarily be applied by researchers from governmental and non-governmental organizations involved in analytical methods development for extraction and determination trace levels of pesticide pollutants in water and food samples.

2. PESTICIDES

Pesticides represented as substances or mixtures designated for destroying and mitigating group of pests such as insects, mammals, microbes and weeds (Huang et al., 2020; Farajzadeh, et al., 2019; Samsidar & Shaarani, 2018). Pesticides of various kinds have been widely employed in agriculture for high yield products during the past decades. Nearly, one-third of the world's agricultural production has been protected by the use of pesticides. Thus, to meet the growing demands of global population, pesticides have been utilized to improve agricultural production (Nsibande & Forbes, 2016).

The use of pesticides is also found in non-agricultural settings, such as the control of industrial vegetation (roadways and railroads), the prevention of pest infestations in buildings, the care of pets, the maintenance of lawns, and the prevention of parasite infections caused by insects that can infect humans.

Pesticides are made up of many different types of chemicals with a wide range of chemical and physical characteristics. Some of these chemicals have varying degrees of toxicity and dispersion, some of which are lasting and some of which disintegrate in the environment. As a result, they may have a negative impact on ecosystems as a whole as well as non-target living things (Nasiri et al., 2020). Organochlorine pesticides (OCPs) such as DDT, whose commercial manufacturing started in 1943, were the first synthetic organic pesticides (Ahmed et al., 2010). It is impossible to prevent pesticides from reaching into contact with the environment due to the widespread use that is constantly expanding. To the extent of our understanding, it is believed that the increase in pesticide usage will continue for many years to come up with the demand for food security of the increasing human population. Additionally, due to the world's rising urbanization and the resulting reduction in land available for farming, society must produce high yield production (Nsibande & Forbes, 2016). In agrochemical products, a wide variety of pesticides, including insecticides, molluscicides, nematicides, rodenticides, avicides, nematicides, herbicides, antimicrobials, repellents, fungicides, algaecides, etc., have been widely utilized (Tudi et al., 2021).

According to Glinski et al. (2018), significant portion of pesticides applied in agricultural areas pass on to surface runoff into lakes, rivers, and streams before leaking into groundwater systems

or volatilizing into the atmosphere. As a result, residues from these compounds may be the major sources of environmental pollution. They may be present in the soil where a crop was grown, as well as in the atmosphere, run-off water from irrigation, ground water, or surface water. As a result, they may also pollute food, food products, and biological systems either directly or indirectly (Samsidar & Shaarani, 2018). Additionally, persistence had been a significant issue, particularly in systems including surface and ground waters.

2.1. Classification of pesticides

Pesticide active components include a wide variety of chemical compounds, including numerous biological agents. The chemical and physical characteristics of pesticides differ from class to class. It is therefore reasonable to classify them according to their characteristics and research their specific groups. Many pesticide structures are quite complicated and cannot be classified into one category. In order to account for the growing range of chemical and biological agents used in pest control or management, systems of classification have arisen. Typically, the chemical composition, target pest species, and method of action of pesticides are used to categorize them (Hassaan & El Nemr, 2020).

2.1.1. Pesticide classification based on chemical composition

The most practical and suitable way to categorize insecticides relies on the chemical composition and how the active components are described. It is a classification kind that offers evidence of effectiveness. Pesticides are classified by their chemical classes into organochlorines, organophosphates, organonitrogen and pyrethroids (Boulanouar et al., 2018).

2.1.1.1. Organonitrogen pesticides

Pesticides with organonitrogen component comprise of a wide range of compounds. In reality, one refers to a particular class of plant protection compounds by the names of the several chemical groups that make up that category. According to the literature (Tankiewicz et al., 2010), carbamates, triazines, and their derivatives are considered to be organonitrogen insecticides. Carbamic acid is used to make carbamate insecticides, which are used to kill or control insects. Carbamates come in a variety of forms, and their toxicity varies according to their molecular structure and operate differently, but in general they degrade more quickly and are less persistent

in the environment than pesticides like pyrethroid, organophosphorous (OPPs), and OCPs. Hence, carbamate pesticides are significant class of pesticides that are frequently employed in agriculture (Wang et al., 2019a; Zhou & Fang, 2015a). The carbamates aldicarb, carbaryl, carbofuran, fenobucarb, methiocarb, thiobencarb, propoxur, and captan are among the most often utilized ones. Insects' brains and nervous systems are damaged by carbamates, which are employed as sprays or baits to kill them. Cockroaches, ants, fleas, crickets, aphids, scale, whiteflies, lace bugs, and mealy bugs can all be killed with carbamates both in and out of the home. According to Zhao et al. (2016), several carbamates have been detected in fruit juice at amounts sufficient to cause the concerns. Gao et al. (2019), further described that exposure to carbamates has the potential to be cancer- and mutagenic-causing, as well as to result in headaches, lightheadedness, or weakness. Additionally, it may result in trembling, stomach cramps, diarrhea, and perspiration. A little rash develops when skin is exposed to carbamates. Long-term exposure may cause weight loss, weakness, and a general feeling of sickness.

Triazine herbicides, which have six membered aromatic ring, prevent photosynthesis and are used for combating weeds. They are heterocyclic ring with three nitrogen atoms replacing carbon-hydrogen units in the benzene ring structure. Tri- signifies three and azine denotes a ring made of nitrogen, which is how the word triazine was created. These herbicides are triazines, which come in symmetrical and asymmetrical forms (Megersa, 2015). Examples of symmetrical triazines are chloro-*s*-triazines (simazine, atrazine, propazine and cyanazine); the thiomethyl-*s*-triazines (ametryn, prometryn, terbutryn) and the methoxy-*s*-triazine (atratone, prometone, secbumetone simetone, and terbumetone). From the isomers of triazine; 1, 3, 5-triazine (also known as *s*-triazines or symmetrical triazines) are the most common and one of the oldest known classes of organic molecules of which about 30% of the herbicides produced. The 6–chloro–*s*–triazine herbicides, including atrazine, cyanazine, propazine and simazine, which inhibit photosynthesis in plants, are the most heavily used pesticides in the world (Megersa, 2015). In soil and water, triazine herbicides are moderately mobile. Triazines are particularly prone to discharge into surface waters and groundwater due to their physicochemical characteristics. Thus, because triazines are very soluble in water, surface and ground waters have become contaminated (Boruah et al., 2021). The increasing use of triazines has effect on the directly treated plants and the entire food chain (Liu et al., 2014) and dangerous to human health causing birth defects, cancers, issues related with the

cardiovascular and reproductive systems, as well as disruptions in hormone function (Mnyandu & Mahlambi, 2021; De Toffoli et al., 2018).

SUHs, a large family of herbicides used extensively in agriculture, have gained quite significant attention all around the world due to their broad spectrum and high herbicidal activity at low dosage application rates (10-40 g ha^{-1}), good crop selectivity, and low mammalian toxicity as a result of their low application rates. There are currently more than 30 commercially available sulfonylurea formulations, making them the second most popular type of herbicide after glyphosate (Gure et al., 2014a). Wheat, vines, rice, citrus, corn, potatoes, tomatoes, and other crops have all been subject to the application of SUHs to suppress weeds (Ghobadi et al., 2015). However, these benefits in controlling herbicides are not without risk to human health and threat to environmental. Depending on the pH, SUHs which are susceptible to contraction of the sulfonylurea linkage degrade in water 10 to1000 times faster than the others (Fenoll et al., 2012). In addition, although the application rates are low, due to relatively high solubility in water and moderate to high mobility, these herbicides may result in leaching into deeper soil and potentially entering surface waters (Kaczyński & Łozowicka, 2017; You & Chen, 2016). Despite the fact that SUHs rapidly decompose in water and soil due to their thermal and chemical instabilities, they still exist in some situations at a trace level (Perreau et al., 2007) and are being detected in surface and ground waters (Chen, et al., 2010). Therefore, the presence of significant levels of pesticide residues in environmental matrices has emerged to pose serious environmental and human health problems (Moreno-gonzález et al., 2017; Shamsipur et al., 2016).

2.1.1.2. Organophosphate pesticides

Esters of phosphoric acid or thiophosphoric acid are organophosphates. Their original chemicals were extremely hazardous to mammals when they were first synthesized in the 1930s and 1940s. Considering their stability, mobility, and long-term impacts on living things, OPPs are among the most hazardous environmental pollutants (Razmi & Jabbari, 2015). OPPs, which are less persistent than OCPs, are frequently the treatment of choice because they effectively, safely, and affordably be managed to a variety of pests and often utilized as pesticides in homes, gardens, and farms (Merdassa et al., 2013). Even at low exposure levels, some OPPs, their metabolites, and breakdown products can have harmful impacts on human health. Among the foods that are most frequently

contaminated with OPPs are fruits and vegetables. The European Union has set maximum residue limits (MRL) of 0.01 mg kg^{-1} for pesticide residues in processed foods and 0.01 - 0.3 mg kg^{-1} for pesticide residues in numerous fruits and vegetables (Xu et al., 2020). According to Heidari et al. (2020), OPPs are known to contribute to or aggravate a number of health issues in humans including cancer, immune system interference, and hormone disturbance. Among these, methidathion, dimethaote, chlorpyrifos, parathion, diazinon, famphur, phorate, terbufos, and malathion are examples of OPPs. In this thesis, two OPPs including dimethaote and methidathion were considered. Dimethaote classified as moderately hazardous (Class II) and methidathion classified as highly hazardous (Class IB) by the World Health Organization, WHO (WHO, 2019).

2.1.1.3. Organochlorine pesticides

OCPs have been employed extensively worldwide in agriculture due to their effectiveness against a range of dangerous insects and their low price (Keswani et al., 2022). Representative compounds in this group include DDT, methoxychlor, dieldrin, chlordane, toxaphene, mirex, kepone, lindane, and benzene hexachloride.

Their low volatility, combined with their extreme stability and probable indiscriminate use in the past, has led to their high persistence both in the environment after application and in organisms after exposure (Zhao et al., 2016). For instance, some OCPs such as DDT, DDE, and DDD have a field half-life of 15 years, whereas aldrin and toxaphene have half-lives of 365 and 9 days, respectively, (Hassaan & El Nemr, 2020). OCPs are stable chemicals that are very persistent and because of their lipophilic nature, they have the potential to accumulate in adipose tissues and bioaccumulate in the food chain (Lucas et al., 2021; Mehlhorn et al., 2022). Their potential for bioaccumulation, chronic toxicity, and possible negative effects on humans and wildlife health resulted to them becoming a global issue of concern (Chen et al., 2022).

During the last decades it became obvious that these chemical compounds are transported through the environment and that critical concentrations have been reached in some areas including those where they have never been used and thus, significant attention has been given to this group of substances on international basis. During the 1970s and 1980s, OCP usage was outlawed in a number of countries. However, because of their efficiency and low cost, organochlorine pesticides like DDT, hexachlorocyclohexane, aldrin, and dieldrin continue to be used routinely in developing

countries like Ethiopia mainly for the control of mosquitoes in health sector (Jayaraj et al., 2016; Mekonen et al., 2017; Merdassa et al., 2015b; Wang et al., 2019b).

2.1.1.4. Pyrethroids pesticides

In order to improve biological performance and environmental stability, in comparison to natural pyrethrins, pyrethroids are synthetic derivatives created by altering specific chemical structures (Ahamad & Kumar, 2023). The natural substances (pyrethrins) that were extracted from plants in the Chrysanthemum genus served as the basis for the pyrethroid class of insecticides (Gao et al., 2018; Niu et al., 2017).

Synthetic pyrethroid pesticides have been used to control agricultural pests all over the world since the 1980s due to their relatively low mammalian toxicity, more efficient at lower concentrations, selective insecticide activity, lack photostability and persistence in the environment compared to the organophosphate, organonitrogen, and OCP classes of pesticides (Niu et al., 2017). Specifically aiming at the receptor location of the voltage-gated sodium channel, pyrethroid herbicides are neurotoxins (Ahamad & Kumar, 2023). By attaching to sodium channels, they render insects incapable of moving and paralyze them excitably (Mao, 2020; Valmorbida et al., 2022). They act in a way that is remarkably similar to natural pyrethrins, which are made from chrysanthemum flowers and are incredibly harmful to fish, aquatic arthropods, and honeybees even at low concentrations (Ahamad & Kumar, 2023) and other researchers found that repeated exposure raises the risk of anaphylaxis and allergic reaction at very low concentrations. Acetamiprid, imidacloprid, lamda-cyhalothrin, and deltametrin are some examples of this group of pesticides.

2.1.2. Pesticides classification based on target pest

Pesticides are categorized as three main groups according to the target organisms, namely, herbicides, insecticides and fungicides (Nasiri et al., 2020). Throughout the world, 40% of applied pesticides are herbicides, 33% are insecticides, while 10% are fungicides and 17% are classified as others (Glinski et al., 2018).

2.1.2.1. Herbicides

Herbicides are the most widely used pesticides class worldwide, followed by insecticides and fungicides. According to Forouzesh et al. (2015), glyphosate, phenoxy acids, benzonitriles, phenylureas, sulfonylureas, triazines, dinitroanalines, amides, thiocarbamates, and uracils are the major chemical groups of herbicides. In this study, the triazines (atraizine, simazine, propazine, prometryn, and cyanazine), phenylurea (linuron) (Bekele & Megersa, 2023a, b; Bekele et al., 2023), and sulfonylureas (chlorsulfuron, chlorimuron-ethyl, flazasulfuron, metsulfuron methyl, niclosulfuron, and prosulfuron) are given particular attention herbicides, since nowadays, they are widely used worldwide for the control of many grasses and most broad leafed weed species in a variety of crops and vegetation. To kill weeds, they are commonly utilized in urban, industrial, and agricultural environments. They are able to offer minimally labor-intensive weed control. Herbicide use has increased crop yield stability, protected crops from unnecessary weed competition, and enhanced the nutritional value of food. To control weeds in agricultural crops, herbicides are typically applied before and after emergence.

The choice of a particular herbicide is typically dependent on a number of factors when the usage of herbicide is needed. In application of pesticides, method being used is the first thing to take into account. Herbicides include application instructions on the label. For instance, a pesticide might be designated for injection or girdling but not basal spraying. Herbicides should only be used for the stated purposes on the label. The relative efficiency of the herbicide in controlling the target plant species, ease of use, relative availability, worker exposure, environmental safety, personal experience, and availability are the second essential factors to take into account when choosing herbicide. It is always necessary to choose herbicide that will effectively control the target species, even though the relative importance of these factors may change depending on the environment and the individual (Heiligmann & Krause, 2021).

2.1.2.2. Insecticides

Insecticides are chemical or biological agents that control insects. Control may result from killing the insect or otherwise preventing it from engaging in behaviors deemed destructive (Nakao & Banba, 2015). Insecticides may be natural or manmade and are applied to target pests in a myriad

of formulations and delivery systems (sprays, baits, slow-release diffusion, *etc*.). The science of biotechnology has, in recent years, even incorporated bacterial genes coding for insecticidal proteins into various crop plants that kill an unsuspecting pests that feed on them (Sparks & Nauen, 2015). Malathion, mercarbam, DDT, aldicarb, carbofuran, pyrethrum and allethrin are classified under this category of pesticides.

2.1.2.3. Fungicides

Fungicides are the type of pesticides used to treat plant diseases caused by different types of fungi, and they have a major impact on improving the productivity and quality of agriculture. The most frequent fungi infections are downy mildew (Plasmopara viticola), powdery mildew (Uncinula necator), and gray mold (Botrytis cinerea) (Bernardi et al., 2022). They can be applied directly to the soil or sprayed over crop fields (Merdassa et al., 2015a). A fungicide with broad-spectrum activity is effective against a number of harmful fungi. Azoxystrobin, capstan, sulfur, and mancozeb are some examples of broad-spectrum fungicides. Some fungicides have a very specific range of activity; mefenoxam, for instance, exclusively works against oomycetes like phytophthora.

2.1.3. Pesticide classification based on mode of action

2.1.3.1. Selective or nonselective pesticides

Selective insecticides are those that kill some organisms in a mixed population while safeguarding the existence of other species. For instance, grasslands, lawns, and gardens all utilize selective herbicides. Some of selective herbicides are employed on croplands including atrazine, 2, 4-D, trifluralin, alachlor, butachlor, fluchloralin, and pendimethalin (Diggle et al., 2003). Whereas non-selective insecticides, such as paraquat, diquat, sodium chlorate, weed oils, and acrolein kill pests regardless of their species. For broad vegetation control on industrial sites, around walkways, fallow land, and in swimming pools and tennis courts, non-selective herbicides like glyphosate (or organic alternatives like nonanoic acid (Slasher) are used. (Diggle et al., 2003).

2.1.3.2. Contact or systemic pesticides

Insecticides that are distributed throughout the plant by absorption are known as systemic insecticides. As a result, the entire plant or some sections thereof turn poisonous to insects. Since

insects that feed on plant tissue must be controlled, this kind of insecticide is crucial. Systemic insecticides can be used against sucking or chewing insects like aphids, caterpillars, and root nematodes. Systemic pesticide has the drawback of killing both harmful and helpful insects. Only the part of the bug that is in touch with the herbicide kills it. Therefore, for effective control, consistent spray coverage and particle size are crucial. Paraquat, diquat, glyphosate, propanil, and petroleum oils are a few examples of typical contact insecticides (Stocka et al., 2011).

2.2. Environmental fates of pesticides and their metabolites

A pesticide natural affinity for one of the four environmental categories; namely, biota, liquid, solid or gaseous affects the pesticides environmental fate (Hassaan & El Nemr, 2020). In general, pesticides have different distribution and persistence patterns in the environment. This behavior can be the measurement of solubility; Henry's Constant (H); the n-octanol/water partition coefficient (K_{OW}) and soil sorption coefficient (K_{OC}). These variables, which are widely known to affect pesticides fate in the environment, are taken into account when determining how each pesticide behaves in the environment. Even though a pesticide is transported in some form through air, soil, and water, its final consequence mostly depends on its persistence and solubility characteristics. They can pass through a variety of processes and pathways after being released into the environment, frequently moving from one medium to another and maintaining a wide range of alterations in the processes (Fig. 1). For instance, a pesticide used directly to a target pest, animal, or plant may have a variety of adverse consequences. It might be absorbed by plants or consumed by animals, insects, worms, or soil-dwelling microbes. In the soil, it may sink and either stick to the granules or travel downhill. The pesticide may potentially evaporate and reach the atmosphere, or it may be metabolized or degraded by microorganisms through chemical routes. It could wash off the surface of the land or leach out of the root zone with rain or irrigation water (Tiryaki & Temur, 2010).

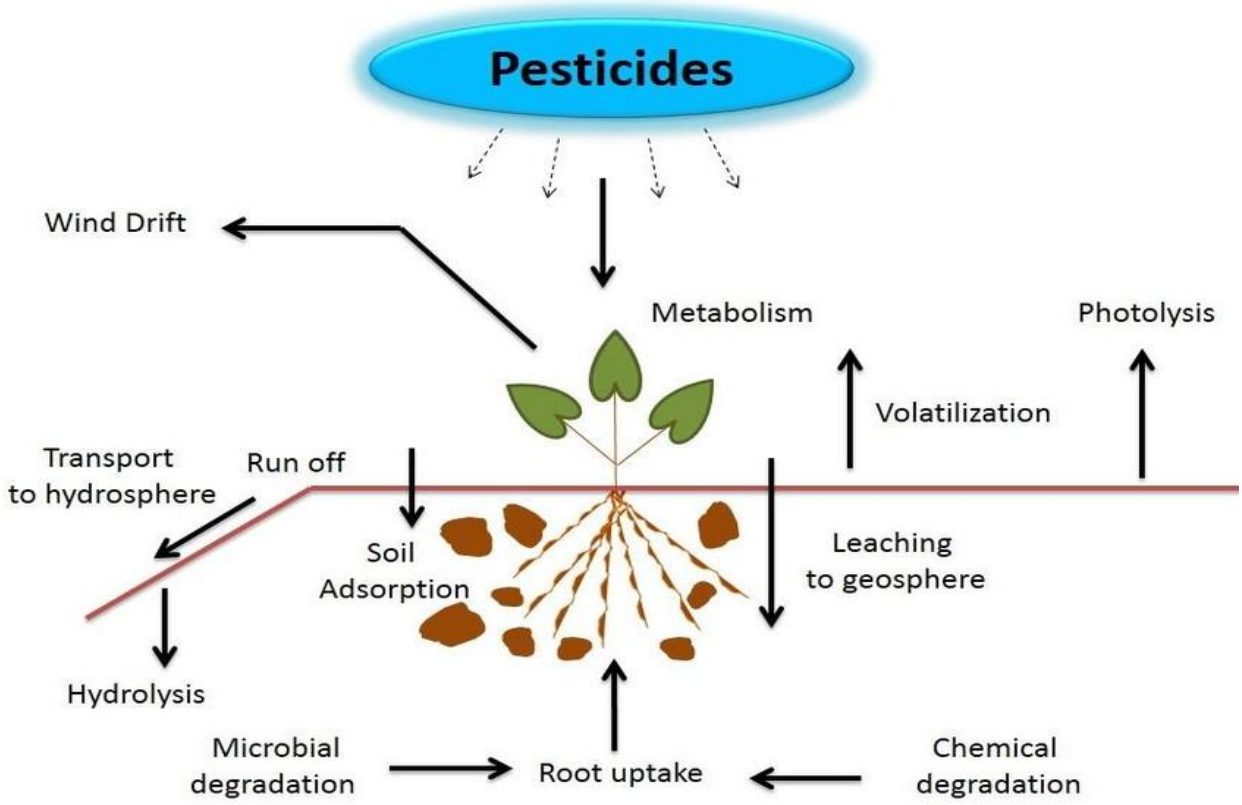


Figure 1. Environmental fates of pesticides and their metabolites.

3. SAMPLE PREPARATION METHODS FOR THE ANALYSIS OF PESTICIDE RESIDUES IN ENVIRONMENTAL AND FOOD MATRICES

Despite their many advantages, pesticides can seriously endanger both human health and the environment if they are used in large quantities. In order to detect their trace residual quantities in food and environmental samples, it will be necessary to develop simple and sensitive analytical techniques. Analytical chemistry has known for decades that it is difficult to accurately determine analytes at the trace level without any pretreatment step. Major advances, in the development of analytical instruments have been made during the last four decades in the area of trace analysis in environmental samples. Despite all the technological breakthroughs in instrument development, instruments are unable to handle samples with complex matrices. To date, the methods used for the analysis of pesticides are mostly based on the chromatographic techniques such as HPLC (Chen et al., 2014) and gas chromatography (GC) (Farajzadeh et al., 2019). These instruments cannot directly handle environmental and food matrices, hence adequate sample preparation or sample pretreatment activities that enrich analytes and reduce interferences must be performed before analysis (Han et al., 2021; Kanu, 2021). Sample preparation is a crucial step in analysis and is often a bottleneck to rapidly obtaining accurate and sensitive results in the determination of trace pollutants (Jain et al., 2021; Ma et al., 2020).

As pesticide pollutants are generally present at low concentrations, they have to be separated from the majority of the components of the accompanying matrix and enriched before analysis. Sample preparation is the process of isolating the desired components from a sample matrix into forms that are appropriate for the analytical procedure. This creates sub-fractions of the original samples that are enriched in all the substances that are important for the analysis while removing other components that could interfere with the analysis. The analytes separated from the matrix are preconcentrated to improve the selectivity, sensitivity, reliability, accuracy and reproducibility of analysis (Nasiri et al., 2020; Wang et al., 2019b). Most often, sample requires sample preparation for one of the following reasons before its identification and/or determination with appropriate analytical instruments. For instance, the technique requires a liquid sample, but the sample is solid; the sample contains interfering matrix components that could cause erroneous or inaccurate readings in the test; and the analyte concentration in the sample is too low for the instrument to detect it. As a result, choosing the best extraction and preconcentration method is crucial for the

accurate measurement of pesticides at trace levels. The analytical techniques used should to be able to measure residues at trace level concentrations and must also be able to offer convincing evidence of both the identity and the concentration of any residues found. The development of new sample preparation processes that are quicker, more efficient, cost effective, and requiring less organic solvent has thus received prior attention in all aspects of separation science research (Tolcha & Megersa, 2018; Zhao et al., 2019).

The majority of instruments available for quantitative studies in biological and chemical sciences are intended for use with liquid samples. For this reason, solid materials to be studied are normally converted to a powder and then dissolved in an appropriate solvent. Depending on the polarity and reactivity of the material, a polar or nonpolar solvent may be selected for this dissolution process. A solvent that can dissolve the full solid sample is selected to guarantee that the analyte has been completely dissolved in the solution of interest. Thus, LLE and SPE were frequently employed when target analytes are not present at the trace level and the sample lacks complex matrices. But in more complex solid and semi solid samples, different efficient extraction methods for trace level pesticides such as Soxhlet, microwave assisted, ultra sound assisted, and pressured liquid extraction (PLE) from soil and sediment (Castiñeira-Landeira et al., 2023; Kunene & Mahlambi, 2020**;** Merdassa et al, 2015b), PLE and super critical fluid extraction (SFE) from onion (Nakamura et al., 2019; Tolcha et al., 2020), LLE and SFE from honey (Baroudi et al., 2021; Lucas et al., 2021; Tolcha et al., 2021; Kujawski et al., 2014; de Pinho et al., 2010), QuChERS from cereals, fruit and vegetables (Mnyandu & Mahalambi, 2021; Ma et al., 2020; Kaczyński et al., 2017; Rai et al., 2016), PLE from milk powder (Mezcua et al., 2007) and matrix solid phase dispersion from vegetables and grain (Liang, et al., 2019) were some of extraction methods employed for soild samples preparation.

3.1. Classical sample preparation techniques for liquid samples

Quite a large number of conventional analytical methodologies including LLE and SPE have been utilized for selective and quantitative extraction of pesticide residues in samples of environmental, biological, food, pharmaceutical, and similar other origins.

3.1.1. Liquid–liquid extraction

LLE, which has been in use for sample preparation for a long time, is one of the simplest and oldest extraction techniques still in use. LLE, often referred to as solvent extraction in which the target analytes are partitioning between two distinct immiscible liquids typically water and organic solvent. The use of LLE is governed by various physicochemical parameters depending on the target analyte to be extracted, which provides information on pH solution, choice of extraction solvent type and volume, and how those choices affect selectivity needed for analyte extraction. Several solvents such as dichloromethane, chloroform, toluene, hexane, ethyl acetate, cyclohexane, and petroleum were used for the analysis of pesticide that depend on the physicochemical characteristics of each pesticide (de Pinho et al., 2010; Timofeeva et al., 2017). LLE has been applied for extraction of multiclass pesticides from honey samples (de Pinho et al., 2010; Kujawski et al., 2014), edible oil (Zhao et al., 2019), milk (Chen et al., 2014) and water samples (Farajzadeh et al., 2015).

The extraction of various sample types and analytes, simplicity, reliability, low cost, and compatibility with majority of the available analytical instruments are the important advantages LLE offers in trace analysis. This method, however, has some inherent drawbacks that can make it expensive or impractical to use: it is labor-intensive, time-consuming, and requires large amounts of organic solvents, many of which are highly toxic to the health of analysists and the environment, in addition to the possibility of emulsions, which may require lengthy extraction steps. Researchers have been emphasizing on developing more effective sample pretreatment and cleanup techniques that can replace or modify this method in recent decades (Samsidar & Shaarani, 2018).

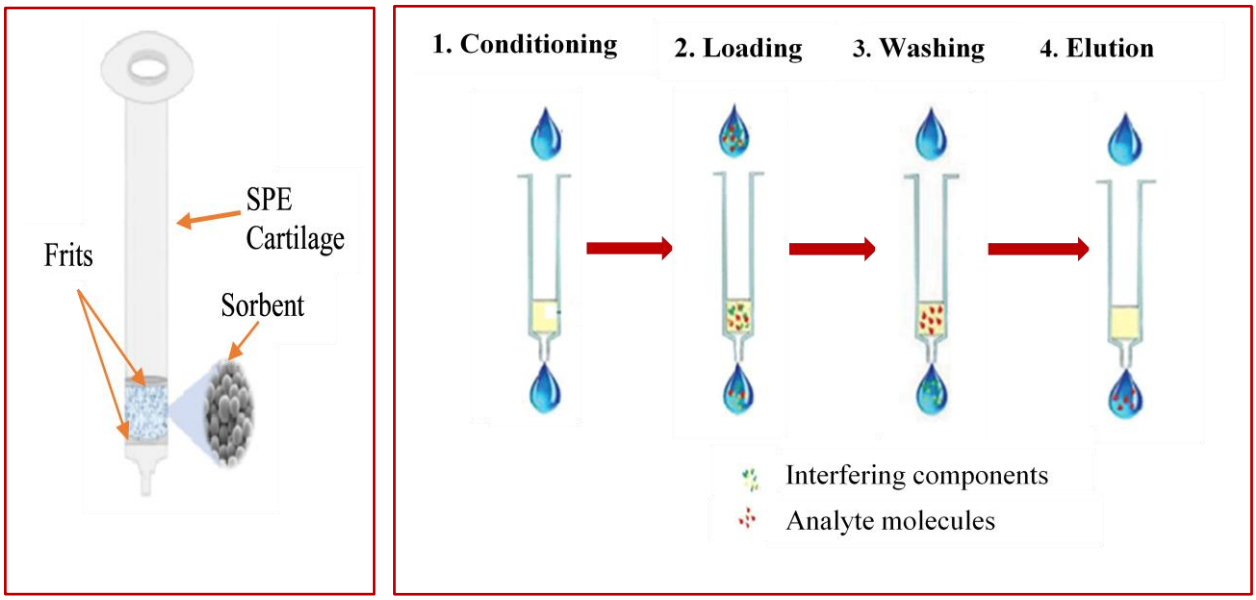
3.1.2. Solid phase extraction

SPE, which was first introduced in early 1970 and developed between 1980 and 1990, is one of the alternate sample preparation techniques to LLE. The SPE procedure relies on distributing the analytes between a solid sorbent packed in a cartridge and a liquid sample that passes through the solid phase. The common components of the solid phase are ion exchangers, organic polymers, and small porous silica particles with or without bound organic phases. Adsorption, partitioning, or ion exchange are the bases for the various extraction mechanisms, depending on the type of

solid phase. Significant works have been performed towards developing and characterizing new formats and advanced sorbent materials, among other SPE related activities, in order to increase the sorbent materials sportive capability, physicochemical stability, and selectivity or specificity for the target analytes (Faraji et al., 2019).

In general, silica or polymer-based sorbents are commonly used to carry out the SPE. The sorbent phase of SPE is largely comparable to that packed into particular HPLC columns. Depending on the fundamental priniciples of standard chromatographic operations, the composition of the SPE sorbent phase can thus change in the bonded functional group based on retention mechanisms, the analyte, the sample conditions, and the solvent employed in conditioning and elution steps. Van der Waals forces, π–π interactions, hydrogen bonds, dipole–dipole interactions, and ion exchange interactions are the most frequently used retention mechanisms in SPE (Zygler et al., 2010). Thus, sorbents can be classified into four groups based on the mechanisms used for retention, including reversed phase (such as C_8 and C_{18} bonded phases) for extraction of hydrophobic analytes from aqueous matrix; normal phase (such as silica, Florisil, alumina, diol, NH_2, and CN bonded phases) for the extraction of polar analytes from non-polar organic solvents, and ion exchange bonded phases (such as sulfonic acid for strong cation exchangers and ammonium for strong anion exchangers) and mixed-mode, which exhibits two or more mode of interaction mechanisms like hydrophobic and ion-exchange functional groups attached to the surface, are used to extract charged analytes from aqueous or non-polar organic samples (Badawy et al., 2022).

SPE has effective purification and preconcentration capacity that can overcome some drawbacks of LLE such as high consumption of sample volume, organic solvent volume and longer extraction time. The lengthy experimental methods required for loading, washing, and elution of the columns as well as the solid phase pores being blocked by large macromolecules are limitations of SPE, even if it consumes less solvent than LLE, as illustrated in Fig. 2. In addition, back pressure problem issue, sorbent ready to use in SPE is relatively expensive, the problem associated to the sample carry over effect which causes increased cost of sample handling and identifying appropriate sorbents during multiclass trace level analysis are all disadvantages of conventional SPE (Han et al., 2021; Liang et al., 2019; Samsidar & Shaarani, 2018).



A B

Figure 2. Schematic diagram of SPE (A) cartilage sorbent and (B) extraction steps (Badawy et al., 2022).

3.2. Microextraction sample preparation techniques for pesticides analysis

Microextraction techniques are typically characterized as non-exhaustive sample preparation techniques that use a very miniature volume of the extracting phase (in the range of μL) relative to the sample volume, implying a high enrichment factor. Additional benefits of microextraction technology include simplicity of use, miniaturization, affordability, and adaptability to a wide range of samples and analytes. These features are crucial for the present development and use of these technologies. The progress of sample extraction technology toward extracting target analytes at micro to nano levels and simplified development has improved extraction effectiveness and decreased resource consumption. SPME, which uses small volume of solid or semi-solid polymeric substance, and liquid phase microextraction (LPME), which uses a small amount of liquid, are both methods for extracting analytes. Both techniques have significant structural variations, yet they have similar characteristics in that they are both microextraction processes (Mirnaghi et al., 2013). Both methods are useful alternatives for sample preparation due to their simplicity, effectiveness, low cost, minimal solvent use and excellent abilities to clean up samples.

Recent investigations have focused on the development of effective, affordable, and miniaturized extraction methods for sample preparation utilizing small amounts of less hazardous solvents in line with the principles of green chemistry (Faraji et al., 2019; Filippou et al., 2017). To this end, DLLME (Ramos et al., 2020), SPME (Füsun et al., 2014), SDME (Yohannes et al., 2016), DSPE (Bekele & Megersa, 2023a) and HF-LPME (Gure et al., 2013; Megersa, 2015) techniques have been developed and widely used as methods of choice for critically minimizing or avoiding the use of organic solvents in sample preparation procedures. However, SPME is expensive, its fiber is brittle and has a short lifespan, it typically has carryover effects, and takes a long time to condition the sorbent (Rezaee et al., 2006; Samsidar & Shaarani, 2018). On the other hand, SDME approaches had shortcomings in drop instability and HF-LPME had poor reproducibility because the membranes cut manually (Samsidar & Shaarani, 2018).

3.2.1. Solid phase microextraction

SPME is the first adsorbent based microextraction which has gained popularity since it was first developed by Pawliszyn's group in the 1990s. This microextraction was developed mainly to minimize the time and extensive consumption of organic solvent and the need for large sample size for sample preparation. It is also the simple sample preparation method with benefits of high speed, sensitive and easy coupling to GC and HPLC. This technique has been considered to be a promising pretreatment method and has been widely used in the areas of the environment analyses (Pei et al., 2018; Yin et al., 2019), food (Souza-Silva et al., 2015), and forensic medicine (Riahi-Zanjani et al., 2018).

The SPME mechanism operates on the idea that analytes are distributed throughout the bulk and the fiber coating. In order to improve the sensitivity, mass transfer velocity, and repeatability of the approach, the stationary phase coated on the fiber is critical (Yin et al., 2019). Many attempts have been undertaken over the past few decades to improve the sensitivity and selectivity for different target analytes, as well as the chemical and thermal constancy of the innovative SPME coating materials. Octadecylsilane (ODS), also called C_{18}, is a kind of surface modified amorphous silica. The C_{18} bonded silica phase is frequently used in SPE to preconcentrate or cleaning nonpolar and medium polar components because it has good separation performance (Günter et al., 2016;

Olisah et al., 2019). Along with SPE, SPME has paid a lot of attention to the use of C_{18} as a hydrophobic stationary phase. OCPs in water samples were found using the C_{18} functionalized SPME fibers. The home made C_{18} composite fiber can be equipped in a fairly constant thickness of 35 mm and a batch of fibers may be manufactured at once using a column assisted process with stainless steel wire as the fiber substrate and the assistance of the GC column (Li et al., 2015). Additionally, the fiber shown outstanding qualities, including exceptional thermal stability, solvent resistance, repeatability, and reproducibility, as well as a wide linear range (Li et al., 2015).

The sample preparation method known as in-tube SPME (IT-SPME) can be used in combination with either HPLC or GC for online analysis. This method is quick, automated, and uses small organic solvents or sample material (Fernández-Amado et al., 2016). For IT-SPME, various extraction phases are often employed, either filled into metal tubes or adhered to the inside of capillaries using covalent or non-covalent interactions. Graphene (G) and GO (Li & Xu, 2015) as well as carbon nano tubes (CNTs) (Jornet-Martínez et al., 2015) have been the subject of numerous investigations on novel materials used as the extraction phase for IT-SPME. The analytical methods selectivity, sensitivity, and extraction effectiveness can all be improved with these materials. The last several years have seen a lot of focus on G and its modifications among these materials. To analyze three of the most prevalent triazines in water samples, a method based on the use of a packed column containing GO supported on aminopropyl silica as sorbent for SPME was developed and optimized. According to de Toffoli et al. (2018), the method revealed that the extraction phase has a high potential for triazines extraction aiming to its physical and chemical properties, including ultrahigh specific surface area, good mechanical and thermal stability, and high fracture strength. For compounds containing aromatic rings in pesticide structures, the electron system in G and its derivatives offers a strong affinity. The application of these materials as a sorbent in packed extraction techniques like IT-SPME, however, does present certain challenges. For instance, the large surface area of G or GO nano sheets raises the extraction pressure and forces the sorbent out of the frits. Despite the many advantages of the traditional SPME, some limitations such as fiber breakage, the sorbent coating losses from the fiber surface during microextraction procedure, swelling of the sorbent in organic solvents, high temperature thermal instability in the thermal desorption procedure were observed (Samsidar & Shaarani, 2018).

3.2.2. Dispersive solid phase extraction

DSPE is a relatively simple and fast sample preparation technique, which was developed by the United States Department of Agriculture (Anastassiades et al., 2003) and has been successfully applied as a method of extraction, isolation, and cleaning in analytic treatment of a wide variety of organic pollutants. DSPE simplifies SPE cleanup, allows more samples to be analyzed at one time, is quite rapid, requires low solvent consumption and also could avoid complex process conditioning and washing, and back pressure problem often encountered in common SPE. DSPE consists of the addition of a solid sorbent, usually silica or polymer based, directly into the sample solution (Han et al., 2014). The dispersion process increases the contact area between the sorbent and the analyte. The sorbents employed in DSPE in the determination of pesticide residues are solids chemically modified by the addition of several chemical compounds that modify their affinities. These modifications ensure the selectivity for the analytes of interest, which allows the maximal retention, minimizing the interferences in the analytical matrix (Sun et al., 2015). After the dispersion, the sorbent is isolated by centrifugation or filtration process. Once the solid phase is isolated, the analytes or interferences adsorbed on the surface of the sorbent could easily be eluted with addition of adequate organic solvents. The removal of potential interferences (clean-up) that might interfere with the subsequent determination of the analytes is accomplished using the DSPE method, which is regarded as a micro and macroscale method of extraction and clean-up used in several analytical procedures (Lin et al., 2016). However, one of the critical steps in DSPE is the selection of the sorbent, and it is necessary to consider chemical and physical characteristics that allow maximal interaction between the sorbent and the analytes, ensuring selectivity of extraction, removal, or preconcentration of the analytes present in analytical matrices (Arnnok et al., 2017). With the additional advantage of low consumption of solvents in the treatment of the sample, DSPE is considered to be a low cost, simple and environmental friendlier technique in comparison with classical techniques such as LLE and SPE (Souza-Silva et al., 2015).

3.2.2.1. Dispersive solid phase extraction based on graphene nanomaterials sorbent

Graphene is a chemical substance (carbon nanomaterial) that contains a single atom thick sheet of honeycomb shaped carbon lattice, possessing a high specific surface area, which makes it extremely good adsorption ability and becoming one of the successful adsorbent of organic compounds (Foschi et al., 2021; Zhang et al., 2017; Zhao et al., 2011). A unique advantage of

graphene over other carbon based nanomaterials is caused by the upper and lower sides of its planes which are accessible for adsorption (Nodeh et al., 2016). However, pure graphene sheets tend to form unrecoverable agglomerates or restore to form graphite through Van der Waals interactions and π-stacking if the sheets are not detached from each other. Therefore, chemical modification of graphene is the most reliable means to synthesize GO which is also important in the preparation of other graphene based sorbent materials (Xu et al., 2020). GO can be produced by chemical oxidation of graphite by strong oxidizing agent using modified Hummer's method and subsequent exfoliation by ultra-sonication (Boruah et al., 2021). This method is commonly used for synthesis of GO due to its low cost, simplicity and scalability. GO appears to be an ultimate sorbent in SPE because as much quantities of oxygen atoms on its surface obtainable from hydroxyl, epoxy, and carboxyl groups (Fig. 3). Because of these functional groups on it, GO has good affinity towards organic compounds containing benzene ring, oxygen and nitrogen functional groups through the formation of a π–π electrostatic interaction, hydrogen bonding and strong π-π stacking interaction (Asadi et al., 2020; Mohammadnia et al., 2020; Sereshti et al., 2014).

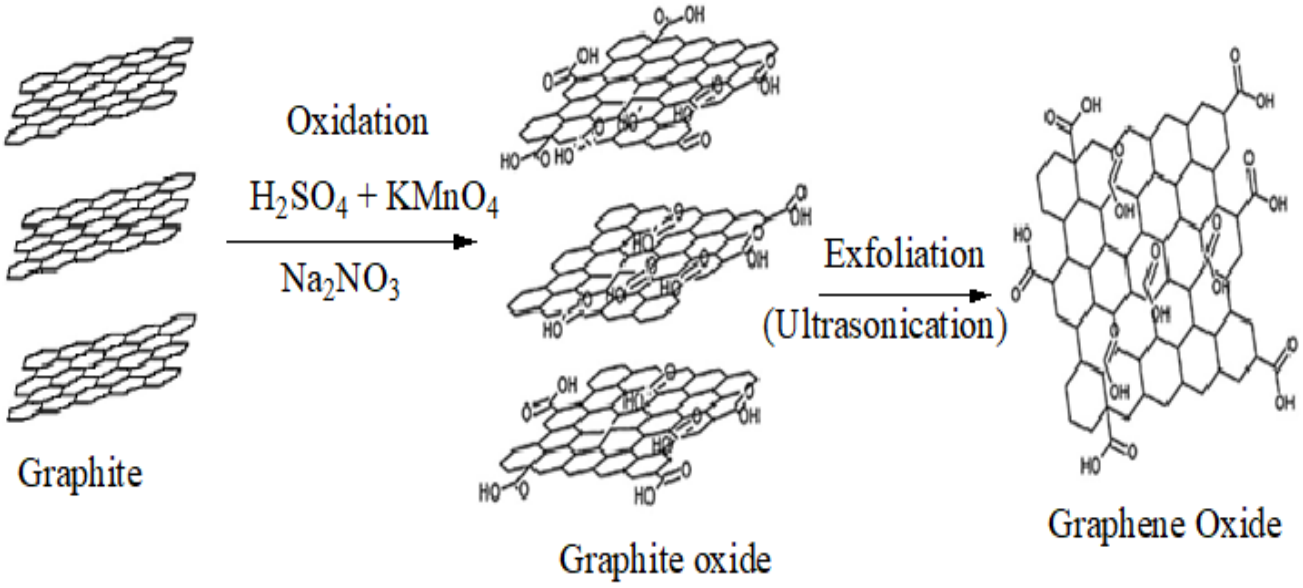


Figure 3. A scheme illustrating synthesis of graphene oxide from graphite (Sereshti et al., 2014).

Nowadays, graphene based materials have been used as adsorbents for the extraction and preconcentration of pesticides such as carbamate (Gao et al., 2019; Song et al., 2014), organophosphorus (Mahpishanian & Sereshti, 2016; Sun et al., 2018), organochlorine

(Mohammadnia et al., 2020), triazine (Zhang et al., 2017; Zhao et al., 2011) and neonicotinoid (Shi et al., 2017). Most of the cited literatures and others use magnetized graphene based nanomaterials and functional composite of GO. Magnetized graphene-based nanomaterial adsorbents, on the other hand, are expensive and labor-intensive to prepare. In addition, the adsorption capacity decreases as the amount of Fe_3O_4 rises because too many magnetic nanoparticles may cover the graphene sheets, necessitating the laborious and time-consuming process of optimizing the amount of Fe_3O_4 nanoparticles (Zhang et al., 2017). The interaction of Fe_3O_4 nanoparticles and GO layer is only by electrostatic or physical adsorption which may be the reason for its leaching out from the GO sheets (Boruah et al., 2017). Hence, the cost effective, efficient, simple and time saving pattern of SPE procedure, *i.e*, DSPE using GO adsorbent can be used for enrichment of trace level inorganic and organic compounds including pesticides in environmental water samples.

GO have been used as the adsorption materials for DSPE. GO nanosheets are negatively charged between pH 2–10 (Deng et al., 2013), which is consistent with the expected results that the ionization of functional groups exist on its surface. Although the substantial electrostatic repulsion caused by these negative charges at this pH range resulted in well dispersed GO in water samples, it makes it difficult to collect when we utilize it as sorbent in DSPE. Also due to the low density of pristine GO, it is exhaustive for separation in centrifugation and the high pressure in filtration**.** Therefore, NaCl was used to neutralize the excessive negative charges and decrease the electrostatic repulsion to carry out the purpose of GO aggregation (Li et al., 2015) as shown in Fig. 4.

As a new mode of SPE, SA-GO-DSPE is performed with the assistance of adding NaCl salt for the purpose of GO aggregation (Fig. 4). This mode was used for efficient extraction of triphenylmethane dyes (malachite green and crystal violet) (Li et al., 2015), heavy metals (Pb, Cd, Bi, and Sb) (Deng et al., 2013), low mass aliphatic amines (dimethylamine and trimethylamine) (Xu et al., 2016) and symmetrical triazine herbicides (atrazine, propazine, prometryn and simazine) (Bekele & Megersa, 2023a) from environmental water samples. Some extraction methods of graphene based nanomaterials sorbent employed for extraction of various types of

pesticide compounds from environmental waters as well as other matrices such as foods are indicated on Table 1.

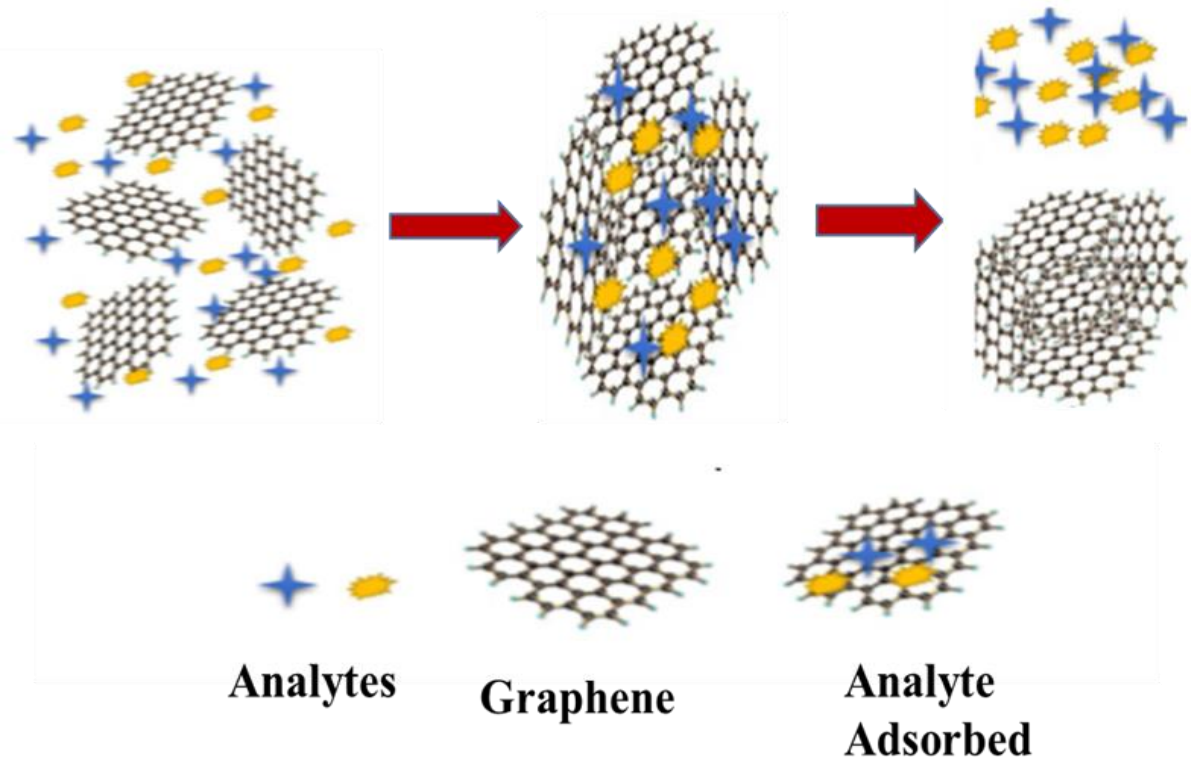


Figure 4. Schematic illustration of the salt assisted GO-DSPE process.

Table 1. Dispersive solid phase extraction based on graphene nano materials sorbent for the extraction and determination of pesticides in water and food samples.

Method	Sorbent	Sorbent Amount (mg)	LOD (µg L^{-1})	Recovery (%)	RSD (%)	Ref.
D-µ-SPE	G/pDA[a]	15	1.5–3	76.8–85.3	0.4–3.0	(Xu et al., 2020)
D-µ-SPE	(GO/Fe_3O_4/TBA)[b]	100	7	88.0-94.0	6.5–7.5	(Mohammadnia et al., 2020)
MSPE	G-CNT- Fe_3O_4[c]	80	1.4–11	76-103	3.9–8.8	(Razmi & Jabbari, 2015)
D-µ-SPE	RGO-TiO_2d	-	2.3-3.3	89–109	1.8-8.3	(Zhou & Fang, 2015a)
MDSPE	IL-MG[e]	40	0.09–0.15	97-101	3.1-6.8	(Zhang et al., 2017)
MSPD	G- Fe_3O_4	100	-	93–106	5.1–7.1	(Chatzimitakos et al., 2019)
MSPE	Fe_3O_4@G-CNPrTEOS[f]	100	0.01–0.6	82-94	1.7-17.6	(Nodeh et al., 2016)
GO-D-µ-SPE	GO	3	1.5	89-110	5.2	(Mahpishanian & Sereshti, 2014)
MSPE	Graphene@SiO_2@Fe_3O_4[g]	20	16-33	90-103	0.5-10	(Wang et al., 2018b)
MSPE	TPN/Fe_3O_4 NPs/GO[h]	11	0.17-1.7	91-102	4.7-9.3	(Moradi Shahrebabak et al., 2019)
DSPE	RGO-ZnO[i]	20	0.01-0.05	75-104	3.7-8.6	(Sun et al., 2015)
SA-GO	GO	1	0.12-0.8	69-96	1.3-9.1	(Bekele & Megersa, 2023a)

G/PDA[a]: Graphene/polydopamine composite; GO/Fe_3O_4/TBA[b]: Magnetic graphene oxide tert-butylamine; G-CNT- Fe_3O_4[c]: Graphene-carbon nanotube-Fe_3O_4; RGO-TiO_2[d]: Reduced graphene oxide-modified TiO_2 nanotube arrays; IL-MG[e]: Ionic liquid-magnetic graphene; Fe_3O_4@G–CNPrTEOS[f]: Magnetic graphene-based cyanopropyltriethoxysilane; Graphene@SiO_2@Fe_3O_4[g]: Magnetic silicon oxide nanocomposite graphene; TPN/Fe_3O_4 NPs/GO[h]: Triazine-based polymeric network modified magnetic nanoparticles/GO; RGO-ZnO[i]: Reduced graphene oxide coated with ZnO nanocomposites.

3.2.3. Dispersive liquid–liquid microextraction

DLLME technique was introduced in 2006 by Assadi and coworkers (Rezaee et al., 2006). The extraction and disperser solvents are rapidly injected into the aqueous sample to generate a cloudy solution, which is the basis for the trace enrichment principle in DLLME. It is a modified form of solvent extraction that has the capacity to maximize enrichment, uses a very small amount of organic solvent, and has significantly lower acceptor to donor phase ratio than other techniques employed for similar purposes (Liu et al., 2022; Wang et al., 2016a).

In general, there are a number of conditions that must be fulfilled before DLLME may be used to extract desired analytes from a given water sample. In the aqueous phase, the dispersing solvent must be completely soluble. For this, acetone, acetonitrile, and methanol are typically utilized. On the other hand, there are a number of requirements that the recommended extracting solvent must adhere to. In order to facilitate phase separation, it must have the ability to extract analytes, be highly soluble in the dispersing solvent while having a very low solubility in water, preferably have good chromatographic behavior and have a density that is significantly different from that of water. In order to achieve a high extraction efficiency, it is crucial to choose the right extracting and dispersion solvents. The extraction and disperser solvents are rapidly injected into the aqueous sample to produce the cloudy solution, which is critical component of trace enrichment principle in DLLME. Extraction equilibrium is quickly maintained because there are strong surface contacts between the droplets of the extraction solvent and the aqueous sample solution (Bekele & Megersa, 2023b; Jain et al., 2021). Furthermore, as a microextraction technique, DLLME provides features such as ease of use, fast, cost-effectiveness, high recovery, and the use of inexpensive, widely accessible laboratory supplies (Huang et al., 2020).

The characteristics of the emulsion interface, surface electrical charge, and Van der Waals forces all affect how stable the small extraction droplets are in the dispersed solution. The effectiveness of demulsification can be significantly influenced by variables like agitation speed, temperature, bulk viscosity, and the presence of contaminants (Ramos et al., 2020). After centrifugation, the extraction solvent is often collected with a microsyringe for chromatographic analysis after sedimenting at the bottom or top of the tube depending on whether the extractants density is higher or lower than that of water. DLLME features include rapidity, ease of application, low cost, high recovery, utilization of cost-effective readily available laboratory equipment, and environmental friendliness (Bernardi et al., 2022). The ratio between analyte concentrations in the organic phase and the sample is known as the preconcentration or enrichment factor (EF). EF is defined as concentration ratio of an analyte in the extraction phase to that in the initial aqueous solution under certain set of circumstances. Calculation of enrichment factor is performed during the DLLME sample preparation process, based on the measured values, equations 1 (Ali et al., 2021; Monireh et al., 2016).

$$EF = \frac{C_{org}}{C_{aq}} \quad (1)$$

where, C_{org} and C_{aq} are the concentration of analyte in the organic phase and aqueous sample, respectively.

Extraction recovery of the analyte (R) is the percentage of the extracted analyte in the organic phase that can be calculated using equation 2; where n_{aq} is the amount of analyte in the aqueous sample prior to extraction (with volume as V_{aq}), and n_{org} is the amount of analyte in organic phase (with volume as V_{org}) (Bú et al., 2020; Farajzadeh et al., 2019).

$$R = \frac{n_{org}}{n_{aq}} x100 = \frac{C_{org}V_{org}}{C_{aq}V_{aq}} x100 = EF x \frac{V_{org}}{V_{aq}} x100 \quad (2)$$

The relative recovery (RR%), which is defined as the ratios of the peak areas of the spiked real water extracts to the peak areas of spiked ultra-pure water extract at the same concentration level which is usually expressed in a percent (Ali et al., 2021), is given by the following relation equation 3.

$$RR\% = \frac{\text{Peak areas of spiked real water extracts}}{\text{Peak areas of spiked ultra pure water extracts}} x100 \quad (3)$$

To study the matrix effect of any type of sample on the accuracy of new developed methods, percent relative recovery can also be calculated using added found (standard addition) method (Jouyban et al., 2020) as given on equation 4.

$$RR\% = \frac{C_{found} - C_{real}}{C_{added}} \quad (4)$$

where C_{found}, C_{real}, and C_{added} are concentration of spiked real sample extract, concentration of unspiked real sample extract and initial concentration spiked (added) respectively.

LOD and LOQ are considered as the analyte minimum concentrations which can be identified and quantified by the method, respectively. The values of LOD and LOQ are very important analytical figure of merit parameters to measure sensitivity of developed methods, including DLLME. Theoretical values estimate of LOD and LOQ can be obtained by using following equations (Bekele & Megersa, 2023a; Zhang et al., 2014a):

$$\mathrm{LOD} = \frac{3\sigma}{m} \quad (5)$$

$$\mathrm{LOQ} = \frac{10\sigma}{\mathrm{m}} \quad (6)$$

where, (σ) is standard deviation of the blank sample analysis and (m) is the slope of calibration curve.

The signal-to-noise ratio can also be used for assessing LOD and LOQ. This method is limited to methods of analysis that display baseline noise. By comparing noticed signals from samples with known low analyte concentrations with those of blank for LOD and LOQ at concentrations that give three and ten times the signal to noise ratio, respectively, one can figure out these analytical values (Castiñeira-Landeira et al., 2023; Tian et al., 2017)

The types and volumes of the extraction and disperser solvent, the pH of the solution, the ionic strength, and extraction time are the main parameters in DLLME that might affect the extraction efficiency and need to be optimized. Since its introduction, the method has commonly been often employed to extract trace level of pesticides, typically from samples of water (Beshana et al., 2022; Cheng et al., 2010; Ramos et al., 2020; Wang et al., 2016a), foods (Carbonell-rozas et al., 2020; Li et al., 2021; Tian et al., 2017), and especially from juices and vegetables (Farajzadeh et al., 2021; Tursen et al., 2021).

3.2.3.1. High density organic solvent based dispersive liquid–liquid microextraction

In high density solvent based DLLME the extraction solvents used are denser than water such as chlorobenzene, carbon tetrachloride, chloroform, tetrachloroethylene, dichloromethane and others (Carbonell-rozas et al., 2020). Dispersive solvents most commonly used are acetone, methanol and acetonitrile can be used as disperser of extraction solvent in sample solution (Wang et al., 2016b). In this technique certain volume of sample solution is placed in a screw cap glass test tube with conical bottom, followed by rapid injection of dispersive solvent containing extraction solvent into the sample solution with a syringe or pipette. Then, the mixture is vortexed, and then a cloudy solution (water/disperser solvent/extraction solvent) is formed in the test tube (Ali et al., 2021).

After that, the surface area between the extraction solvent and aqueous phase (sample) is infinitely large, thereby, transfer of analyte from aqueous phase to organic phase is fast. Subsequently, equilibrium state is achieved quickly, realized in a very short extraction time, which is the remarkable advantage of DLLME compared with those of other techniques. Finally, the dispersed fine particles of extraction phase are sedimented at the bottom of conical test tube through centrifugation and certain volume of the sedimented phase is injected into chromatographic system using microsyringe for further analysis (Fig. 5). High density solvent based DLLME has been employed for extraction of various types of pesticide compounds from environmental waters as well as other matrices such as foods (Table 2).

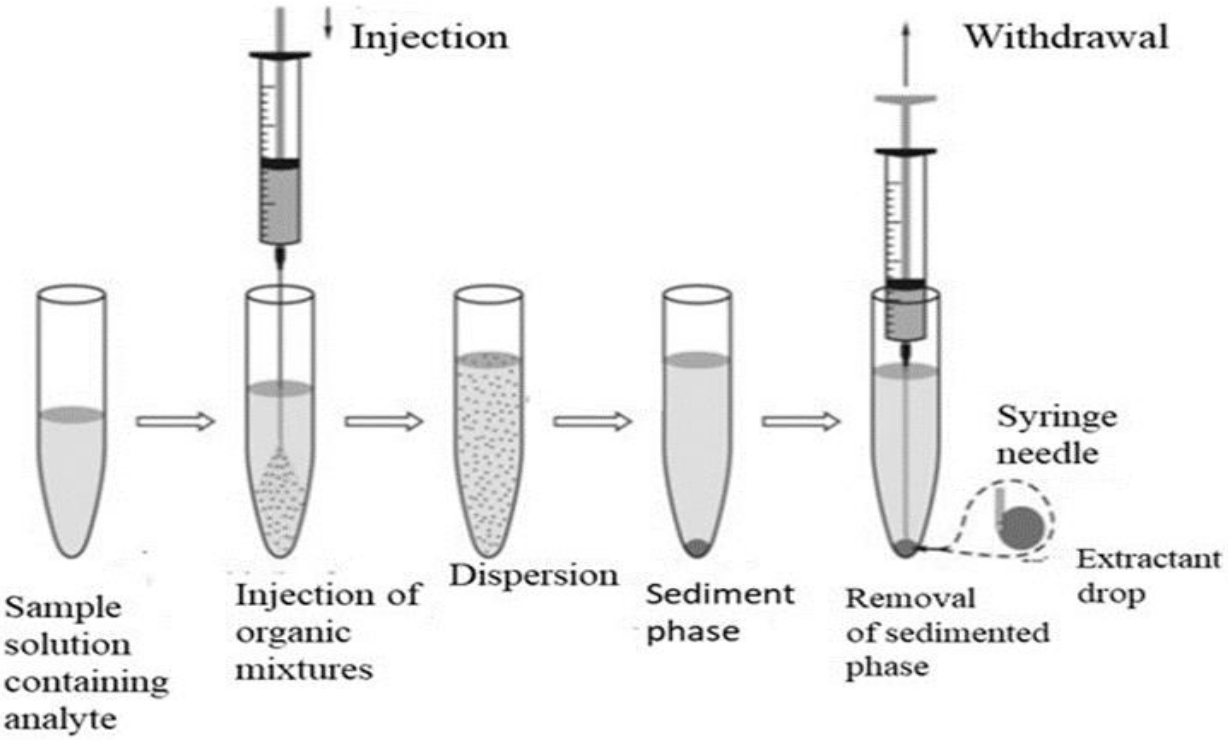


Figure 5. Schematic diagram of high density solvent based DLLME procedure (Rezaee et al., 2006).

Table 2. High density solvent based DLLME for extraction and determination of pesticides in water and food samples.

Method	Extraction solvent	Extraction solvent volume (µL)	LOD (µg L^{-1})	Recovery (%)	RSD (%)	Ref.
DLLME	Chlorobenzene	60	0.2-0.3	85-104	3.3-6.5	(Wu et al., 2010)
DLLME	Chloroform	800	2-9	72-110	1-9.8	(Gure et al., 2013)
DLLME	Carbon tetrachloride	60	0.2-2	62-108	2.1 - 6.4	(Zhang et al., 2014b)
MDSPE-DLLME	Trichloroethane	35	0.15-0.36	87-101	2 - 6	(Ali & Mohebbi, 2018)
DLLME	Chloroform	100	0.0014-0.008	71-93	5-11	(Wang et al., 2016b)
SE-VA-DLLME[a]	Dichloromethane	200	0.38-0.83	93-99	3.8 -7.1	(Tursen et al., 2021)
DLLME	Chloroform	40	0.005-0.02	80-114	0.7-8.7	(Tolcha & Megersa, 2018)
DLLME	Dibromoethane	55	0.05-0.08	87-116	2.2-7.1	(Ali et al., 2021)
VA-DLLME	Chloroform	250	0.7–0.8	95–119	5.1–9.8	(Muckoya et al., 2020)
DLLME	Dichloromethane	2000	3.1-6.6	80-99	0.6-6.3	(Carbonell-rozas et al., 2020)
VA-DLLME	Chlorobenzene	310	1.2-2.5	83-92	2.6-5.6	(Wang et al., 2018a)
DLLME	Dichloromethane	300	0.2-7.8	81-120	3.4-7.5	(Bodur et al., 2020)
DLLME	Acetonitrile	50	0.9-5	86-112	0.9-4.2	(Nisha et al., 2021)

SE-VA-DLLME[a]: Surfactant-emulsified vortex-assisted DLLME method

3.2.3.2. Low density organic solvent based solvent demulsification dispersive liquid-liquid microextraction

Lower density organic solvents such as 1-octanol, toluene, *n*-hexane, and cyclohexane are employed as extraction solvents, whereas acetone, methanol, and acetonitrile can be used as dispersants (Pirsaheb et al., 2015). With the exception of the type of organic solvent and the organic phase floating on the surface of an aqueous sample, this technique's basic principles are the same as those of high-density organic solvent-based DLLME. A microsyringe is used to remove the organic layer for further chromatographic analysis. Many researchers have recently tried to use low toxicity solvents in DLLME that have densities lower than water (Yan et al., 2016). However, these solvents are difficult to collect, as meniscus formed at the top of a conical tube is very difficult to withdraw (Bernardi et al., 2022). Different approaches have been proposed

to solve this problem, including solvent terminated DLLME (ST- DLLME) (Bernardi et al., 2022; Guo & Lee, 2012; Tolcha et al., 2013; Yan et al., 2016). Instead of centrifugation, demulsification is taken to separate the organic and aqueous phases after extraction. A certain amount of organic solvent was added as demulsifier to change the interfacial adhesion between the organic and aqueous phases, surface tension and Van der Waal's forces, then the emulsion system was found to be broken and extraction solvent droplets will be collected.

Utilizing alcohols such as 1-octanol, 1-dodecanol, and undecanol as extractor solvents is one of the most important advantages of ST-DLLME. In general, alcohols are less hazardous than the chlorinated solvents that are frequently employed in DLLME. Along with attempting to apply the fundamental components of green analysis, another significant benefit is the decrease in extraction time and energy consumption due to the elimination of the centrifugation step, use in field processing, and automated analysis (Yan et al., 2016).

3.2.3.3. Low density solvent based dispersive liquid-liquid microextraction surface floating organic droplet

Different modes of DLLME for preconcentration of multiple pesticide residues in a variety of food matrices such as vegetables and fruit juices have become widely used in recent years as a result of the replacement of traditional sample preparation techniques with DLLME (Bernardi et al., 2022; Heidari et al., 2020; Wang et al., 2018a; Wang et al., 2017; Wang et al., 2016a). Although conventional DLLME, which commonly uses chlorinated solvents, has many advantages including ease of use, speed, affordability, high recoveries, and a high enrichment factor (Rai et al., 2016), the widespread use of halogenated solvents as extraction solvents has been constrained by their toxicity, which is also linked to human cancers. As a result, usage of chlorinated solvents has been outlawed globally and gradually been replaced with more environmentally friendly solvents that are lighter in density than water to address these shortcomings (Ago et al., 2023). DLLME-SFOD is a recently proposed method used for the speciation and preconcentration of trace amounts of analytes (Pirsaheb et al., 2017). Moreover, in DLLME-SFOD technique, collection of extraction solvent seems to be interesting and easy task for the extraction of multiresidue compounds as the dispersed fine particles of extraction phase droplets are floats on surface of samples in extraction

vessels after centrifugation as illustrated in Schematic diagram Fig. 6, which show extraction procedures that were followed for enrichment of multiclass pesticide residues from different fruit juice samples utilizing DLLME-SFOD analytical method (Bekele & Megersa, 2023b). After its development, numerous research works have been reported (Table 3) and the performance of DLLME-SFOD was illustrated by extraction of different pesticides in environmental water and different food samples (Chen et al., 2020; Jouyban et al., 2020; Pirsaheb et al., 2017; Ramos et al., 2020). This method has been regarded as a powerful, sensitive, high performance, environment friendly, rapid and cost-effective liquid-phase micro extraction method.

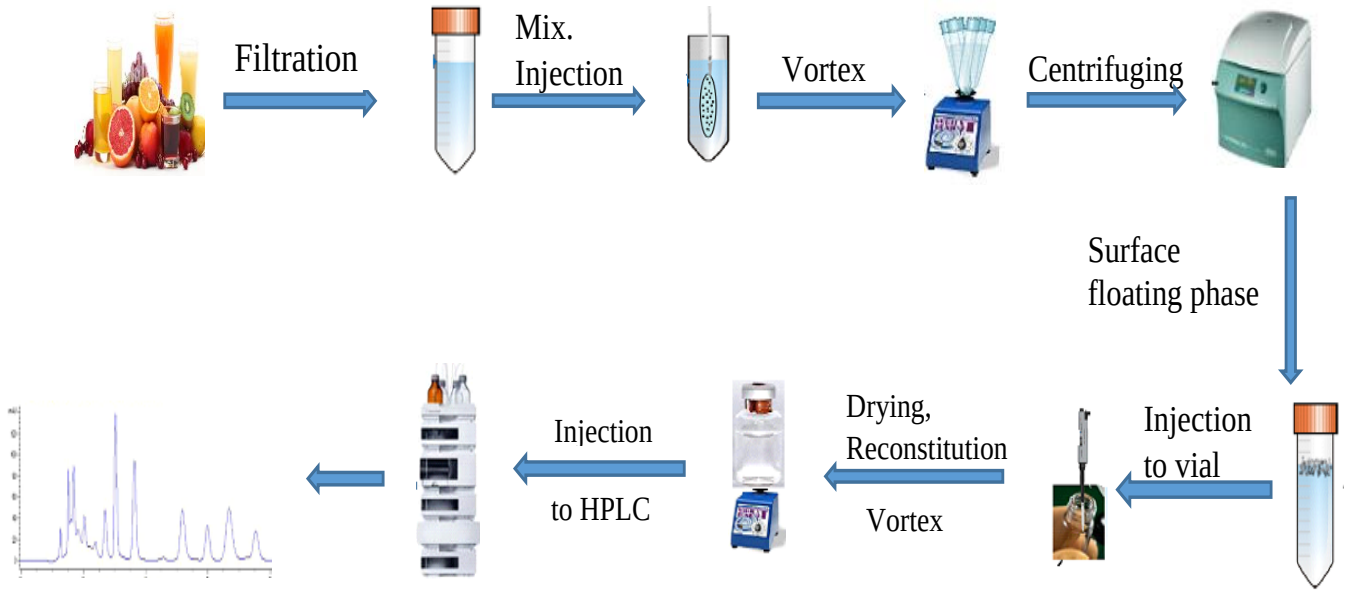


Figure 6. Schematic diagram of low density solvent based DLLME-SFOD procedure

Table 3. Low density solvent based DLLME surface floating organic droplet for the extraction and determination of pesticides in water and food samples.

Method	Extraction solvent	Extraction solvent volume (µL)	LOD (µg L^{-1})	Recovery (%)	RSD (%)	Ref.
VA-DLLME[a]	1-Undecanol	300	1.5–3	58–66	4.4–5.1	(Sharafi et al., 2015)
DLLME-SFO	Hexadecane	30	0.08–0.44	90-108	2-5.8	(Gao et al., 2018)
DES-SFO-DLLME[b]	DES	63	1.3-3.9	74-89	3.8-6.9	(Jouyban et al., 2020)
SDLLME-SFO[c]	1-Undecanol	30	1-2	58-67	4.3–6.8	(Pirsaheb et al., 2015)
VA-DLLME	1-Dodecanol	200	5.6-17.4	77-99	1.3–9.8	(Chen et al., 2020)
UA-DLLME-SFO	1-Undecanol	150	2–8	68–88	4.5-9	(Monireh et al., 2016)
DLLME-SFO	Hexadecane	180	60-300	91-109	1-10	(Ramos et al., 2020)
DES-UALLME	DES	408	0.07- 0.10	87–117	5.1-7.8	(Heidari et al., 2020)
SD-DLLME	1-Dodecanol	15	0.24-0.81	79-105	1.2-6	(Yan et al., 2016)
DLLME-SFOD	Nonanoic acid	82	2.7-4.9	84-93	2.3-8.6	(Huang et al., 2020)
DLLME	Hexane	47	0.17-0.41	79-97	2.2-10.5	(Beshana et al., 2022)
EPA-DLLME[d]	Toluene	125	0.03–0.24	76–116	0.33–5.52	(Ago et al., 2023)
DLLME-SFOD	Toluene	100	0.02-0.05	87-99	1.8-10.8	(Bekele & Megersa, 2023b)
SD-DLLME[e]	Octanol	100	0.03-0.06	70-117	3-20	(Bernardi et al., 2022)

VA-DLLME[a]: Vortex-assisted DLLME; DES-SFO-DLLME[b]: Deep eutectic solvent-based surface floating organic DLLME; SDLLME-SFO[c]: Sonication DLLME based on the SFOD; EPA-DLLME[d]: Effervescent powder assisted floating organic solvent-based DLLME; SD-DLLME[e]: Solvent-based demulsification DLLME.

3.2.4. Salting out assisted liquid–liquid extraction

Homogeneous liquid–liquid microextraction (HLLME) has also been used to extract target analytes by achieving complete miscibility of the two phases (water-miscible extraction solvent and aqueous sample solution). In HLLME technique, phase separation can be based on salting-out phenomenon (with addition of a salt), temperature, pH, or ion-pair formation (Farajzadeh et al., 2018; Sherin et al., 2022). Among various HLLME techniques, SALLE came into practice to initiate the separation between the low volume organic solvent and aqueous sample mixture at high salt concentrations as illustrated in Fig.7. In this extraction technology, the salt addition is the main operation for achieving the increased extraction of the target analytes in the aqueous sample because it can increase ionic strength of the solution and decrease the solubility of weak

electrolytes in water by the salting out effect. It is comparatively a novel procedure that uses salt for phase separation and a water miscible organic solvent as an extraction solvent (Mayara et al., 2020). Relative to LLE, it does not require prolonged mechanical shaking and vacuum distillation to obtain good extraction efficiency and high enrichment factors as the two phases exist in a miscible state. Solvents used are much more environmental-friendly, and also lower quantities are needed for the extraction procedure.

The most popular methods, DLLME, are limited to the use of nonpolar, water immiscible solvents with low dielectric constants and poor extraction efficiency of polar organic and inorganic compounds (Heydari et al., 2017; Tighrine et al., 2019). However, SALLE is an efficient extraction method for polar to moderately polar organic compounds, was made feasible by using more polar and water miscible organic extraction solvents like acetonitrile, isopropanol, acetone, ethanol, methanol, among others (Chirfa et al., 2020; Pasupuleti et al., 2020; Sherin et al., 2022; Bekele et al., 2023). Three factors mainly considered when selecting a salt for the salting-out process. These including the solubility of the salt in the organic solvent should be negligible; the solubility of the salt in the water should be high in order to maximize the interaction with water molecules and the salting out capacity should follow the hofmeister Series. Hofmeister series is the empirical order of ions based on their ability to precipitate hydrophilic substances; i.e., $Mg^{2+} > Ca^{2+} > Sr^{2+} > Ba^{2+} > Li^{+} > Na^{+} > K^{+} > Rb^{+} > Cs^{+}$ for cations and citrate > tartrate > $SO_4^{2-} > C_2H_3O^{2-} > C1^{-} > NO_3^{-} > ClO_3^{-} > I^{-} > SCN^{-}$ for cations (Salis & Ninham, 2014).

Generally, SALLE is a simple and powerful preconcentration method that reduces the extraction time, cost, solvent consumption and exposure to the organic solvent in comparison to other traditional LLE sample preparation techniques. The SALLE method produce extracts with solutes in organic solvent that may be evaporated and reconstituted with appropriate solvent for preconcentration and analysis by HPLC or GC (Akram et al., 2017; Lucas et al., 2021a). On the other hand, in the SALLE methods extraction solvents are compatible with the majority of analytical instruments, particularly chromatographic ones, making it possible to directly inject the extract into these methods of analysis (Lakew et al., 2022; Niu et al., 2017). SALLE has been used successfully to analyze pesticides in foods (Akram et al., 2017; Farajzadeh et al., 2018; Rashidipour et al., 2018), biological matrices (Niu et al., 2017), and environmental waters

(Alemayehu et al., 2017; Gure et al., 2014b; Wen et al., 2013). Researchers put a lot of work into making the method automated and high throughput during development step to reduce processing time and chemicals required (Mohd et al., 2018; Nováková & Vlčková, 2009). Though various pretreatment technologies have been developed, the method of SALLE has still been widely used, since it integrates sample clean-up, preconcentration and extraction in a single step and shares the advantages of the sample pretreatment technique gained from QuEChERS (Du et al., 2014; Pasupuleti et al., 2020). Some application SALLE and its efficiency validations for extraction of pesticides and their determination in water and food samples are indicted in Table 4.

Table 4. Salting out liquid-liquid extraction of pesticides and their determination in water and food samples.

Matrix	Extraction solvent and volume (mL)	Salt type and amount (g)	LOD ($\mu g\ L^{-1}$)	Recovery (%)	RSD (%)	Ref.
Water and fruit juice	Acetonitrile , 1	$NH_4)_2SO_4$, 1.60	0.4-13	72-115	0.3-13	(Gure et al., 2014b)
Water	Acetonitrile, 1.5	NaCl, 1.4	2-2.8	76–107	0.67-10	(Alemayehu et al., 2017)
Water	Acetonitrile, 1	NaCl, 1.4	0.02-0.19	63-115	0.3-9.8	(Teju et al., 2017)
Saline water	Acetonitrile, 2	NaCl, 0.6	1.5-61	74-110	1.8-9.8	(Niu et al., 2017)
Fruit juices	Isopropanol, 0.2	Na_2SO_4 , 2	0.22–0.5	86-96	2-7	(Farajzadeh et al., 2018)
Water and vegetables	Acetonitrile, 2.5	NaCl, 3.5	20	80-96	3.5-7.5	(Rashidipour et al., 2018)
Honey	Acetonitrile, 1	Acetate buffer, 0.3	0.25ng/g	98-104	5.3-13.7	(Giroud et al., 2019)
Water and edible seeds	Acetonitrile, 2	NaCl, 2.9	0.3	82-95	<6.5	(Rouhollah & Rezvan, 2021)
Fruit and vegetables	Acetonitrile, 4.8	NaCl/Na_2SO_4, 1, in the ratio of 0.87	30	78-92	1.1-4.9	(Akram et al., 2017)
Water	Acetonitrile, 6	NaCl, 6.4	0.34-5.0	87-95	2-7	(Farajzadeh et al., 2015)
Cow milk	Acetonitrile, 1	$MgSO_4$, 2	0.58–2.6	86–109	1.97-8.0	(Bekele et al., 2023)

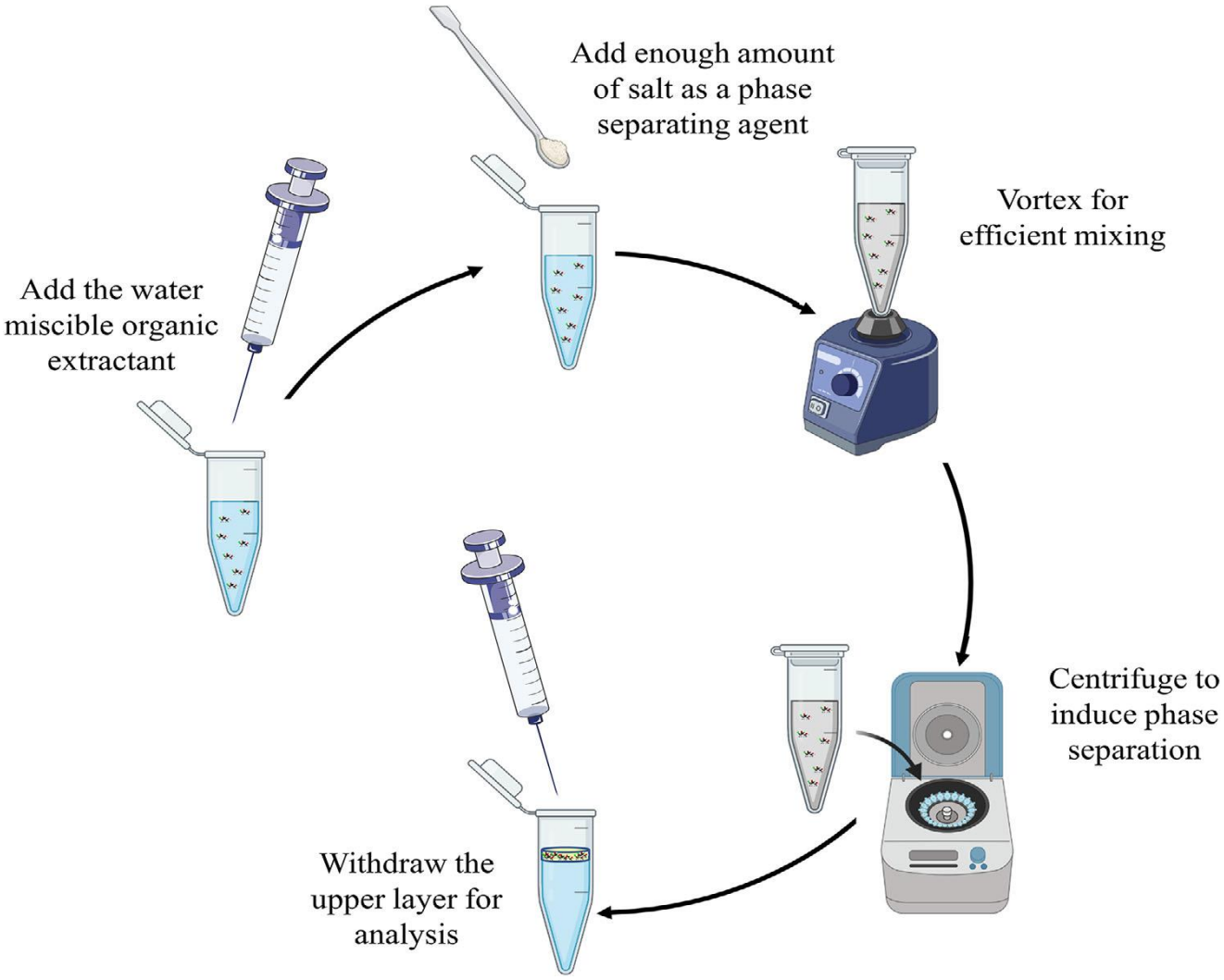


Figure 7. Schematic diagram of the SALLE procedure.

3.3. Separation and analysis of pesticide residues in this study

It is important to monitor pesticide concentrations utilizing sensitive techniques for parts per million (ppm) or parts per billion (ppb) levels. Identification and quantification of hundreds of pesticides with substantially different physicochemical properties in widely different types of matrices are of paramount importance because the major task of analytical discipline is to provide precise and affordable methods for the analysis of pollutant residues in both the environmental and food matrices.

There has been a clear shift from the use of persistent insecticides such as organochlorine compounds to more polar and readily degradable pesticides such as organophosphorus pesticides and organonitrogen pesticides, which are universally applied because of their ready availability, wide range of efficacy, and being less stable in the environment than organochlorine compounds (Nisha et al., 2021; Maciej et al., 2010). The widespread use of herbicides, including triazines, chlorophenoxy acids, phenylureas, and more recently, sulfonylureas, is another significant trend. The majority of these pesticides are low volatile, polar, and/or thermos labile substances that require derivatization in order to be directly amenable to GC. Most of very polar pesticides can be efficiently separated with reversed phase liquid chromatography (RPLC) without a preceding laborious derivatization step. Hence, since the introduction of RPLC equipped with a suitable/robust ultra violet (UV) (Rawat et al., 2023; Rejczak & Tuzimski, 2017; Wang et al., 2019a; Zeiadi et al., 2020) or fluorescence detector (Tejada-casado et al., 2018) was used in the field of pesticide residue analysis, which occurred around 1980s LC became rapidly adopted as a viable technique complementary to GC for the determination of various classes of polar pesticides. The wide application range, long-term stability, ease of use, low cost and improved selectivity of DAD makes the UV detection mode most widely used in residue analysis.

The recent introduction into the market of robust LC–MS instruments provides a new way for analyzing polar pesticides more efficiently. It is ideal to render a highly accurate and reliable determination that encompasses both qualitative and quantitative estimation of pesticide residues. LC–MS/MS is the preferred technique for the vast majority of pesticides because of the polar nature of the majority of pesticides currently in use, and especially of their metabolites (Giroud et al., 2019; Grimalt & Dehouck, 2016; Lachat & Glauser, 2018). These instrumentation will

encounter difficulties because of the complex nature of sample pretreatments, the requirement for highly trained professionals, and the expensive cost of equipment. In comparison, rapid pesticide analysis techniques, such as immunoassays, spectroscopic analysis, and electrochemical techniques, result in pesticide detection approaches that are relatively straightforward and extremely sensitive, even if the accuracy and precision of these rapid methods are not as good as those of chromatography instrumental procedures (Campanale et al., 2021).

Thus, in the area of polar to moderately polar pesticide residues analysis, there is a high demand and remarkable developments in extending the applicability of RPLC coupled with DAD for simple, cost effective and efficient analytical techniques for the fast and accurate determination of such target analytes in different matrices. Therefore, in the present research work, HPLC-DAD was utilaized for quantitative determination of selected pesticide residues in environmental water and food samples.

4. APPLICATIONS IN THIS THESIS

Microextraction methods are promising scientific solution for trace level pesticide residues enrichment and extraction from environmental water and food samples matrix, since such methods are simple, sensitive, selective and environmentally friendly techniques. In this research work, SA-GO-DSPE, DLLME-SFOD, SALLE, and HD-DLLME were developed, optimized and validated for sensitive and selective analysis of trace level pesticide pollutants utilizing HPLC-DAD as a reliable alternative in regular laboratory for determinations of selected *s*-triazine pesticides and SUHs in environmental water samples; and multiclass pesticides in fruit juice and cow milk samples were applied in this thesis as presented in the following papers.

Paper 1. Green analytical method based on salt assisted graphene oxide dispersive solid phase extraction of symmetrical triazine herbicides in environmental water samples for liquid chromatographic determination.

Paper 2. Fast surface floating organic droplets based dispersive liquid–liquid microextraction for trace enrichment of multiclass pesticide residues from different fruit juice samples followed by high performance liquid chromatography–diode array detection analysis.

Paper 3. A Highly selective analytical method based on salt assisted liquid-liquid extraction for trace level enrichment of multiclass pesticide residues in cow milk for quantitative liquid chromatographic analysis.

Paper 4. Development of simple and cost effective dispersive liquid–liquid microextraction analytical method for extraction and preconcentration of multiresidue herbicides in environmental water samples.

5. REFERENCES

Ago, K. A., Kitte, S. A., Chirfa, G., & Gure, A. (**2023**). Effervescent powder assisted floating organic solvent based dispersive liquid–liquid microextraction for determination of organochlorine pesticides in water by GC–MS. *Heliyon*, *9*(1), e12954.

Ahamad, A., & Kumar, J. (**2023**). Pyrethroid pesticides: An overview on classification, toxicological assessment and monitoring. *Journal of Hazardous Materials Advances*, *10*, 100284.

Ahmadi-jouibari, T., Pasdar, Y., & Fattahi, N. (**2017**). Continuous sample drop flow microextraction followed by high performance liquid chromatography for determination of triazine herbicides from fruit juices. *Analytical Methods*, *9*(6), 980–985.

Akram, S., Sultana, B., & Asi, M. R. (**2017**). Salting out assisted liquid–liquid extraction and reverse phase high performance liquid chromatographic monitoring of thiacloprid in fruits and vegetables. *Separation Science and Technology*, *6395*, 1–10.

Alemayehu, Y., Tolcha, T., & Megersa, N. (**2017**). Salting out assisted liquid–liquid extraction combined with hplc for quantitative extraction of trace multiclass pesticide residues from environmental waters. *American Journal of Analytical Chemistry*, *8*, 433–448.

Ali, M., Kiavar, L., & Pezhhanfar, S. (**2021**). Development of a method based on dispersive liquid–liquid microextraction followed by partial vaporization of the extract for ultra preconcentration of some pesticide residues in fruit juices. *Journal of Chromatography A*, *1653*, 462427.

Ali, M., & Mohebbi, A. (**2018**). Development of magnetic dispersive solid phase extraction using toner powder as an efficient and economic sorbent in combination with dispersive liquid–liquid microextraction for extraction of some widely used pesticides in fruit juices. *Journal of Chromatography A*, *1532*, 10–19.

Anastassiades, M., Maštovská, K., & Lehotay, S. J. (**2003**). Evaluation of analyte protectants to improve gas chromatographic analysis of pesticides. *Journal of Chromatography A*, *1015*(1–2), 163–184.

Antônio, R. D. S., Carolina, A., Guerra, S., Andrade, M. B. De, & Nishi, L. (**2021**). Application of graphene nanosheet oxide for atrazine adsorption in aqueous solution: synthesis, material characterization, and comprehension of the adsorption mechanism. *Environmental Science and Pollution Research*, *28*, 5731–5741.

Arnnok, P., Patdhanagul, N., & Burakham, R. (**2017**). Dispersive solid phase extraction using polyaniline modified zeolite NaY as a new sorbent for multiresidue analysis of pesticides in food and environmental samples. *Talanta, 164*, 651–661.

Asadi, M., Sereshti, H., & Rashidi Nodeh, H. (**2020**). Development of magnetic dispersive micro solid phase extraction using lanthanum phosphate nanoparticles doped on magnetic graphene oxide as a highly selective adsorbent for pesticide residues analysis in water and fruit samples. *Research on Chemical Intermediates, 46*(5), 2789–2803.

Badawy, M. E. I., El-Nouby, M. A. M., Kimani, P. K., Lim, L.W., & Rabea, E. I. (**2022**). A review of the modern principles and applications of solid phase extraction techniques in chromatographic analysis. *Analytical Sciences, 38*, 1457-1487.

Baroudi, F., Al-Alam, J., Delhomme, O., Chimjarn, S., Al-Ghech, H., Fajloun, Z., & Millet, M. (**2021**). Liquid–liquid extraction procedure for nonvolatile pesticides determination in acacia honey as environmental biomonitor. *Euro-Mediterranean Journal for Environmental Integration, 6*(3), 1–8.

Barriada-Pereira, M., Concha-Graña, E., González-Castro, M. J., Muniategui-Lorenzo, S., López-Mahía, P., Prada-Rodríguez, D., & Fernández-Fernández, E. (**2003**). Microwave assisted extraction versus soxhlet extraction in the analysis of 21 organochlorine pesticides in plants. *Journal of Chromatography A, 1008*(1), 115–122.

Bekele, H., & Megersa, N. (**2023a**). Green analytical method based on salt assisted graphene oxide dispersive solid phase extraction of symmetrical triazine herbicides in environmental water samples for liquid chromatographic determination. *International Journal of Environmental Analytical Chemistry*, 1–17. https://doi.org/10.1080/03067319.2022.2161377

Bekele, H., & Megersa, N. (**2023b**). Fast surface floating organic droplets based dispersive liquid–liquid microextraction for trace enrichment of multiclass pesticide residues from different fruit juice samples followed by high performance liquid chromatography diode array detection analysis, *Separation Science Plus, 6*(8), 1–12.

Bekele, H., Yohannes, W., & Megersa, N. **(2023**). A Highly selective analytical method based on salt assisted liquid–liquid extraction for trace level enrichment of multiclass pesticide residues in cow milk for quantitative liquid chromatographic analysis. *International Journal of Analytical Chemistry, 2023*, 1-13.

Bernardi, G., Kemmerich, M., Machado, F. F., Prestes, O. D., Adaime, M. B., & Zanella, R. (**2022**). Multiresidue determination of fungicides in wine by solvent demulsification dispersive liquid–liquid microextraction and ultra high performance liquid chromatography – tandem mass spectrometry. *Food Analytical Methods*, 2026–2035.

Beshana, S., Hussen, A., Leta, S., & Kaneta, T. (**2022**). Dispersive liquid–liquid microextraction coupled with microfluidic paper based analytical device for the determination of organophosphate and carbamate pesticides in the water sample. *Analytical Sciences*, *38*(10), 1359–1367.

Bhattacharyya, S., Poi, R., Baskey Sen, M., Mandal, S., Hazra, D. K., & Karmakar, R. (**2023**). Efficient fabrication of pH modified graphene nano adsorbent for effective determination and monitoring of multiclass pesticide residues in market fresh vegetables by GC–MS. *Journal of Food Composition and Analysis*, *118*, 105153.

Bodur, S., Borahan, T., Ates, N., & Bakırdere, S. (**2020**). Sensitive determination of acetochlor, alachlor, metolachlor and fenthion utilizing mechanical shaking assisted dispersive liquid–liquid microextraction prior to gas chromatography–mass spectrometry. *Bulletin of Environmental Contamination and Toxicology*, *105*(3), 460–467.

Boonchiangma, S., Ngeontae, W., & Srijaranai, S. (**2012**). Determination of six pyrethroid insecticides in fruit juice samples using dispersive liquid–liquid microextraction combined with high performance liquid chromatography. *Talanta*, *88*, 209–215.

Boruah, P. K., Darabdhara, G., & Das, M. R. (**2021**). Polydopamine functionalized graphene sheets decorated with magnetic metal oxide nanoparticles as efficient nanozyme for the detection and degradation of harmful triazine pesticides. *Chemosphere*, *268*, 129328.

Boruah, P. K., Sharma, B., Hussain, N., & Das, M. R. (**2017**). Magnetically recoverable Fe_3O_4/graphene nanocomposite towards efficient removal of triazine pesticides from aqueous solution: Investigation of the adsorption phenomenon and specific ion effect. *Chemosphere*, *168*, 1058–1067.

Boulanouar, S., Mezzache, S., Combès, A., & Pichon, V. (**2018**). Molecularly imprinted polymers for the determination of organophosphorus pesticides in complex samples. *Talanta*, *176*, 465–478.

Bú, K., Kosti, I., & Hrouzková, S. (**2020**). Development of a multiresidue QuEChERS–DLLME fast GC–MS method for determination of selected pesticides in yogurt samples. *Food Analytical Methods*, *13*, 829–1841.

Campanale, C., Massarelli, C., Losacco, D., Bisaccia, D., Triozzi, M., & Uricchio, V. F. (**2021**). The monitoring of pesticides in water matrices and the analytical criticalities: A review. *Trends in Analytical Chemistry*, *144*, 116423.

Campos do Lago, A., da Silva Cavalcanti, M. H., Rosa, M. A., Silveira, A. T., Teixeira Tarley, C. R., & Figueiredo, E. C. (**2020**). Magnetic restricted access carbon nanotubes for dispersive solid phase extraction of organophosphates pesticides from bovine milk samples. *Analytica Chimica Acta*, *1102*, 11–23.

Carabias-Martínez, R., Rodríguez-Gonzalo, E., Herrero-Hernández, E., & Hernández-Méndez, J. (**2004**). Simultaneous determination of phenyl and sulfonylurea herbicides in water by solid phase extraction and liquid chromatography with UV diode array or mass spectrometric detection. *Analytica Chimica Acta*, *517*(1–2), 71–79.

Carbonell-rozas, L., García-campaña, A. M., Lara, F. J., & Iruela, O. (**2020**). Capillary liquid chromatography as an effective method for the determination of seven neonicotinoid residues in honey samples. *Journal of Separation Science*, *43*, 3847–3855.

Castiñeira-Landeira, A., Vazquez, L., Gonzalez-Leirado, H., Llompart, M., & Dagnac, T. **(2023)**. Ultrasound-assisted extraction followed by liquid chromatography coupled to tandem mass spectrometry for the simultaneous determination of multiclass herbicides in soil. *Analytical and Bioanalytical Chemistry*, 1–13.

Chatzimitakos, T. G., Karali, K. K., & Stalikas, C. D. (**2019**). Magnetic graphene oxide as a convenient nanosorbent to streamline matrix solid phase dispersion towards the extraction of pesticides from vegetables and their determination by GC–MS. *Microchemical Journal*, *151*, 104247.

Chen, D., Zhang, Y., Miao, H., Zhao, Y., & Wu, Y. (**2015**). Determination of triazine herbicides in drinking water by dispersive micro solid phase extraction with ultra high performance liquid chromatography high resolution mass spectrometric detection. *Journal of Agricultural and Food Chemistry*, *63*(44), 9855–9862.

Chen, D., Zhao, Y., Miao, H., & Wu, Y. (**2015**). A novel dispersive micro solid phase extraction using PCX as the sorbent for the determination of melamine and cyromazine in milk and milk powder by UHPLC–HRMS/MS. *Talanta*, *134*, 144–152.

Chen, L., Chen, J. F., Guo, Y., Li, J., Yang, Y., Xu, L., & Fu, F. (**2014**). Study on the simultaneous determination of seven benzoylurea pesticides in Oolong tea and their leaching characteristics during infusing process by HPLC–MS/MS. *Food Chemistry*, *143*, 405–410.

Chen, L., Wu, J., & Huang, X. (**2019**). Multiple monolithic fibers modified with a molecularly imprinted polymer for solid phase microextraction of sulfonylurea herbicides based on boron nitrogen interaction. *Microchimica Acta*, *186*(7), 470.

Chen, X., Panuwet, P., Hunter, R. E., Riederer, A. M., Bernoudy, G. C., Barr, D. B., & Ryan, P. B. (**2014**). Method for the quantification of current use and persistent pesticides in cow milk, human milk and baby formula using gas chromatography tandem mass spectrometry. *Analytical Technologies in the Biomedical and Life Sciences*, *970*, 121–130.

Chen, X., Gao, L., Hu, Y., Luan, L., Tong, R., Zhang, J., & Wang, H. (**2022**). Distribution, sources, and ecological risk assessment of HCHs and DDTs in water from a typical coal mining subsidence area in Huainan, China. *Environmental Science and Pollution Research*, *29*, 59985–59995.

Chen, Z., Li, Q., Yang, T., Zhang, Y., He, M., Zeng, H., Mai, X., Liu, Y., & Fan, H. (**2020**). Sequential extraction and enrichment of pesticide residues in Longan fruit by ultrasonic assisted aqueous two phase extraction linked to vortex assisted dispersive liquid–liquid microextraction prior to high performance liquid chromatography analysis. *Journal of Chromatography A*, *1619*, 460929.

Cheng, J., Zhou, Y., Zuo, M., Dai, L., & Guo, X. (**2010**). Application of dispersive liquid–liquid microextraction and reversed phase high performance liquid chromatography for the determination of two fungicides in environmental water samples. *International Journal of Environmental Analytical Chemistry*, *90*(11), 845–855.

Chirfa, G., Merdassa, Y., & Gure, A. (**2020**). Salting out assisted liquid–liquid extraction for analysis of caffeine and nicotinic acid in coffee by HPLC–UV/VIS detector. *Journal of Analysis and Testing*, *4*(4), 298–306.

Council of the European Communities. **(1998)**. Council Directive 98/83/EC on the quality of water intended for human consumption. *Official Journal of the European Communities*, L330, 32–54.

de Oliveira Arias, L. J., D. O., Rocha, C. B., Kupski, L., Barbosa, S. C., & Primel, E. G. (**2021**). Salting out induced liquid–liquid microextraction : an environmentally friendly approach to preservative determination in food samples. *Food Analytical Methods*, *14*, 1625–1636.

de Pinho, G. P., Neves, A. A., de Queiroz, M. E. L. R., & Silvério, F. O. (**2010**). Optimization of the liquid-liquid extraction method and low temperature purification (LLE–LTP) for pesticide residue analysis in honey samples by gas chromatography. *Food Control*, *21*(10), 1307–1311.

de Toffoli, A. L., Fumes, B. H., & Lanças, F. M. (**2018**). Packed in-tube solid phase microextraction with graphene oxide supported on aminopropyl silica: Determination of target triazines in water samples. *Journal of Environmental Science and Health - Part B*, *53*(7), 434–440.

Deng, D., Jiang, X., Yang, L., Hou, X., & Zheng, C. (**2013**). Organic solvent free cloud point extraction like methodology using aggregation of graphene oxide. *Analytical Chemistry*, *86*(1), 758–765.

Diggle, A. J., Neve, P. B., & Smith, F. P. (**2003**). Herbicides used in combination can reduce the probability of herbicide resistance infinite weed populations. *Weed Research*, *43*(5), 371–382.

Du, D., Dong, G., Wu, Y., Wang, J., & Gao, M. (**2014**). Salting out induced liquid–liquid microextraction based on the system of acetonitrile/magnesium sulfate for trace level quantitative analysis of fluoroquinolones in water, food and biological matrices by high performance liquid chromatography with a fluorescence detector. *Analytical Methods*, *6*, 6973–6980.

European Commission. **(2005)**. Regulation (EC) No. 396/2005 of the European Parliament and of the Council on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. *Official Journal of the European Communities*, L70, 1–16.

Fang, G., Chen, J., Wang, J., He, J., & Wang, S. (**2010**). N-Methylimidazolium ionic liquid-functionalized silica as a sorbent for selective solid phase extraction of 12 sulfonylurea herbicides in environmental water and soil samples. *Journal of Chromatography A*, *1217*(10), 1567–1574.

Fang, G., Wang, X., & Wang, S. **(2010)**. Multiwalled carbon nanotubes as SPE adsorbents for simultaneous determination of seven sulfonylurea herbicides in environmental water by LC–MS/MS. *Chromatographia*, *72*(5–6), 403–409.

Faraji, M., Yamini, Y., & Gholami, M. (**2019**). Recent advances and trends in applications of solid phase extraction techniques in food and environmental analysis. *Chromatographia*, *82* (8), 1207–1249.

Farajzadeh, M. A., Hessamaddin, S., Ali, M., & Mohammad, R. A. M. (**2019**). Combination of a modified quick, easy, cheap, efficient, rugged, and safe extraction method with a deep eutectic solvent based microwave assisted dispersive liquid–liquid microextraction: Application in extraction and preconcentration of multiclass pesticides. *Journal of Separation Science*, *42*(6), 1273–1280.

Farajzadeh, M. A., Barazandeh, S., Pezhhanfar, S., Reza, M., & Mogaddam, A. (**2023**). Graphene modified magnetic nanoparticles for analyzing some pesticides through magnetic dispersive solid phase extraction and dispersive liquid–liquid microextraction followed by GC–MS determination. *Food Analytical Methods*, *16*, 177–189.

Farajzadeh, M. A., Feriduni, B., & Afshar Mogaddam, M. R. (**2015**). Development of counter current salting out homogenous liquid–liquid extraction for isolation and preconcentration of some pesticides from aqueous samples. *Analytica Chimica Acta*, *885*, 122–131.

Farajzadeh, M. A., Mohebbi, A., Mogaddam, A. R. M., Davaran, M., & Norouzi, M. (**2018**). Development of salt induced homogenous liquid–liquid microextraction based on iso–propanol/sodium sulfate system for extraction of some pesticides in fruit juices. *Food Analytical Methods*, *11*, 2497–2507.

Farajzadeh, M. A., Pirmohamadlou, A., & Sattari Dabbagh, M. (**2021**). Extraction and preconcentration of some pesticides in vegetable and fruit juice samples using SA@$CaCO_3$ sorbent combined with dispersive liquid–liquid microextraction. *Food Analytical Methods*, *14*(11), 2395–2407.

Fenoll, J., Hellín, P., Sabater, P., Flores, P., & Navarro, S. (**2012**). Trace analysis of sulfonylurea herbicides in water samples by solid phase extraction and liquid chromatography–tandem mass spectrometry. *Talanta, 101*, 273–282.

Fernández-Amado, M., Prieto-Blanco, M. C., López-Mahía, P., Muniategui-Lorenzo, S., & Prada-Rodríguez, D. (**2016**). Strengths and weaknesses of in-tube solid phase microextraction: A scoping review. *Analytica Chimica Acta, 906*, 41–57.

Filippou, O., Bitas, D., & Samanidou, V. (**2017**). Green approaches in sample preparation of bioanalytical samples prior to chromatographic analysis. *Journal of Chromatography B, 1043*, 44–62.

Forouzesh, A., Zand, E., Soufizadeh, S., & Samadi Foroushani, S. (**2015**). Classification of herbicides according to chemical family for weed resistance management strategies an update. *Weed Research, 55*(4), 334–358.

Foschi, M., Capasso, P., Maggi, M. A., Ruggieri, F., & Fioravanti, G. (**2021**). Experimental design and response surface methodology applied to graphene oxide reduction for adsorption of triazine herbicides. *ACS Omega, 6*(26), 16943–16954.

Füsun, O. P., Levent, P., Tuğberk, N. D., Can, A., Hasan, E. E., Yalcınkaya, E. E., & Hayati T¨urkmen, F. N. (**2014**). A novel polythiophene ionic liquid modified clay composite solid phase microextraction fiber: Preparation, characterization and application to pesticide analysis. *Analytica Chimica Acta, 895*, 37-45.

Gammon, D. W., Aldous, C. N., Carr, W. C., Sanborn, J. R., & Pfeifer, K. F. (**2005**). A risk assessment of atrazine use in California: Human health and ecological aspects. *Pest Management Science, 61*(4), 331–355.

Gao, T., Wang, J., Wu, Q., Wang, C., & Wang, Z. (**2019**). A graphene oxide based composite for solid phase extraction of carbamate pesticides from vegetables. *Food Analytical Methods, 13*, 690–698.

Gao, Y., Xu, C., Liu, S., & Sun, P. (**2018**). Determination of seven pyrethroid pesticides in liquid milk by dispersive liquid–liquid microextraction based on the solidification of a floating organic droplet followed by GC. *Chromatographia, 81*(3), 539–544.

Gebremichael, S., Birhanu, T., & Tessema, D. A. (**2013**). Analysis of organochlorine pesticide residues in human and cow's milk in the towns of Asendabo, Serbo and Jimma in South Western Ethiopia. *Chemosphere, 90*(5), 1652–1657.

Ghobadi, M., Yamini, Y., & Ebrahimpour, B. (**2015**). Extraction and determination of sulfonylurea herbicides in water and soil samples by using ultrasound assisted surfactant enhanced emulsification microextraction and analysis by high performance liquid chromatography. *Ecotoxicology and Environmental Safety*, *112*, 68–73.

Giroud, B., Bruckner, S., Straub, L., Neumann, P., Williams, G. R., & Vulliet, E. (**2019**). Trace level determination of two neonicotinoid insecticide residues in honey bee royal jelly using ultrasound assisted salting out liquid–liquid extraction followed by ultra high performance liquid chromatography–tandem mass spectrometry. *Microchemical Journal*, *151*, 104249.

Glinski, D. A., Purucker, S. T., Van Meter, R. J., Black, M. C., & Henderson, W. M. (**2018**). Analysis of pesticides in surface water, stemflow, and throughfall in an agricultural area in South Georgia, USA. *Chemosphere*, *209*, 496–507.

González-Rodríguez, M. J., Liébanas, F. J. A., Frenich, A. G., Vidal, J. L. M., & López, F. J. S. (**2005**). Determination of pesticides and some metabolites in different kinds of milk by solid phase microextraction and low pressure gas chromatography–tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, *382*(1), 164–172.

Grimalt, S., & Dehouck, P. (**2016**). Review of analytical methods for the determination of pesticide residues in grapes. *Journal of Chromatography A*, *1433*, 1–23.

Günter, A., Balsaa, P., Werres, F., & Schmidt, T. C. (**2016**). Influence of the drying step within disk based solid phase extraction both on the recovery and the limit of quantification of organochlorine pesticides in surface waters including suspended particulate matter. *Journal of Chromatography A*, *1450*, 1–8.

Guo, L., & Lee, H. K. (**2012**). Low density solvent based ultrasound assisted emulsification microextraction and on-column derivatization combined with gas chromatography–mass spectrometry for the determination of carbamate pesticides in environmental water samples. *Journal of Chromatography A*, *1235*, 1–9.

Gure, A., Lara, F. J., Megersa, N., Garc, A. M., & Olmo-iruela, M. (**2013**). Hollow fiber liquid phase microextraction combined with capillary HPLC for the selective determination of six sulfonylurea herbicides in environmental waters. *Journal of Separation Science*, *36*, 3395–3401.

Gure, A., Lara, F. J., Megersa, N., Olmo-iruela, M., & García-campaña, A. M. (**2014a**). Dispersive liquid–liquid microextraction followed by capillary high performance liquid chromatography for the determination of six sulfonylurea herbicides in fruit juices. *Food Analytical Methods*, *7*, 1465–1473.

Gure, A., Lara, F. J., Moreno-gonzález, D., Megersa, N., Olmo-iruela, M., & García-campaña, A. M. (**2014b**). Salting out assisted liquid–liquid extraction combined with capillary HPLC for the determination of sulfonylurea herbicides in environmental water and banana juice samples. *Talanta*, *127*, 51–58.

Hamilton, D. J., Ambrus, Á., Dieterle, R. M., Felsot, A. S., Harris, C. A., Holland, P. T, Katayama, A., Kurihara, N., Linders, J., Unsworth, J., & Wong. S. S. **(2003)**. Regulatory limits for pesticide residues in water (IUPAC technical report). *Pure Applied Chemistry*, *75*, 1123–1155.

Han, F., Zhou, D. B., Song, W., Hu, Y. Y., Lv, Y. N., Ding, L., Zheng, P., Jia, X. Y., Zhang, L., & Deng, X. J. (**2021**). Computational design and synthesis of molecular imprinted polymers for selective solid phase extraction of sulfonylurea herbicides. *Journal of Chromatography A*, *1651*, 462321.

Han, L., Sapozhnikova, Y., & Lehotay, S. J. (**2014**). Streamlined sample cleanup using combined dispersive solid phase extraction and invial filtration for analysis of pesticides and environmental pollutants in shrimp. *Analytica Chimica Acta*, *827*, 40–46.

Hassaan, M. A., & El Nemr, A. (**2020**). Pesticides pollution: Classifications, human health impact, extraction and treatment techniques. *Egyptian Journal of Aquatic Research*, *46*(3), 207–220.

Hazra, D. K., & Purkait, A. (**2019**). Role of pesticide formulations for sustainable crop protection and environment management: A review. *Journal of Pharmacognosy and Phytochemistry*, *8*(2), 686–693.

Heidari, H., Ghanbari-rad, S., & Habibi, E. (**2020**). Optimization deep eutectic solvent based ultrasound assisted liquid-liquid microextraction by using the desirability function approach for extraction and preconcentration of organophosphorus pesticides from fruit juice samples. *Journal of Food Compostion and Analysis*, *87*, 103389.

Heiligmann, R. B., & Krause, D. (**2021**). Relative effectiveness of herbicides commonly used to control woody vegetation in forest stands. School of Natural Resources, Ohio State University Report, (0051 herbicide relative effectivenes.pdf (osu.edu).

Heydari, R., Hosseini, M., & Rezaeepour, R. (**2017**). Semi automated salt assisted liquid–liquid extraction coupled to high performance liquid chromatography to determine three aromatic hydrocarbons in aqueous samples. *Journal of the Iranian Chemical Society*, *14*(8), 1691–1698.

Huang, X., Du, Z., Wu, B., Jia, L., Wang, X., & Jing, X. (**2020**). Dispersive liquid–liquid microextraction based on the solidification of floating organic droplets for HPLC determination of three strobilurin fungicides in cereals. *Food Additives & Contaminants: Part A*, *37*(8), 1279-1288.

Hussen, A., Westbom, R., Megersa, N., Mathiasson, L., & Björklund, E. (**2007).** Selective pressurized liquid extraction for multiresidue analysis of organochlorine pesticides in soil. *Journal of Chromatography A*, *1152* (1-2), 247–253.

Jain, R., Kumari, A., & Khatri, I. (**2021**). Simple and rapid analysis of acetaminophen in human autopsy samples by vortex assisted dispersive liquid–liquid microextraction thin layer chromatography image analysis. *Separation Science Plus*, *4*, 92–100.

Jayaraj, R., Megha, P., & Sreedev, P. (**2016**). Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. *Interdisciplinary Toxicology*, *9*(3–4), 90–100.

Jornet-Martínez, N., Serra-Mora, P., Moliner-Martínez, Y., Herráez-Hernández, R., & Campíns-Falcó, P. (**2015**). Evaluation of carbon nanotubes functionalized polydimethylsiloxane based coatings for in-tube solid phase microextraction coupled to capillary liquid chromatography. *Chromatography*, *2*(3), 515–528.

Jouyban, A., Ali, M., Reza, M., & Mogaddam, A. (**2020**). In matrix formation of deep eutectic solvent used in liquid phase extraction coupled with solidification of organic droplets dispersive liquid-liquid microextraction ; application in determination of some pesticides in milk samples. *Talanta*, *206*, 120169.

Kaczyński, P., & Łozowicka, B. (**2017**). One-step QuEChERS based approach to extraction and cleanup in multiresidue analysis of sulfonylurea herbicides in cereals by liquid chromatography–tandem mass spectrometry. *Food Analytical Methods*, *10*(1), 147–160.

Kanu, A. B. (**2021**). Recent developments in sample preparation techniques combined with high performance liquid chromatography : A critical review. *Journal of Chromatography A*, *1654*, 462444.

Keswani, C., Dilnashin, H., Birla, H., Roy, P., Tyagi, R. K., Singh, D., Rajput, V. D., Minkina, T., & Singh, S. P. (**2022**). Global footprints of organochlorine pesticides: a pan-global survey. *Environmental Geochemistry and Health*, *44*, 149–177.

Kujawski, M. W., Bargańska, Z., Marciniak, K., Miedzianowska, E., Kujawski, J. K., Ślebioda, M., & Namieśnik, J. (**2014**). Determining pesticide contamination in honey by LC–ESI–MS/MS–Comparison of pesticide recoveries of two liquid–liquid extraction based approaches. *LWT - Food Science and Technology*, *56*(2), 517–523.

Kunene, P. N., & Mahlambi, P. N. (**2020**). Optimization and application of ultrasonic extraction and Soxhlet extraction followed by solid phase extraction for the determination of triazine pesticides in soil and sediment. *Journal of Environmental Chemical Engineering*, *8*(2), 103665.

Kunene, P. N., & Mahlambi, P. N. (**2019**). Development and application of SPE–LC–PDA method for the determination of triazines in water and liquid sludge samples. *Journal of Environmental Management*, *249*, 109415.

Lachat, L., & Glauser, G. (**2018**). Development and validation of an ultra sensitive UHPLC–MS/MS method for neonicotinoid analysis in milk. *Journal of Agricultural and Food Chemistry*, *66*(32), 8639–8646.

Lakew, A., Megersa, N., & Chandravanshi, B. S. (**2022**). Quantitative extraction of multiclass antibiotic residues in cow milk utilizing salting out assisted liquid–liquid extraction followed by liquid chromatographic determination. *International Journal of Environmental Analytical Chemistry*, 1–19.

Li, G., Zhang, X., Zhang, L., Xu, S., & Li, C. (**2015**). Salt assisted graphene oxide dispersive solid phase microextraction for sensitive detection of malachite green and crystal violet by HPLC. *Chromatographia*, *78*(15), 979–985.

Li, J., Xu, W., Wang, X., Wu, F., Wang, L., Feng, J., Wang, Z., & Zhang, H. (**2021**). Ionic liquid based dispersive liquid–liquid microextraction followed by dispersive solid phase extraction coupled with HPLC–DAD for the determination of sulfonylurea herbicides in soymilk samples. *Journal of Liquid Chromatography and Related Technologies*, *44*(13–14), 663–673.

Li, Y., & Xu, H. (**2015**). Development of a novel graphene/polyaniline electrodeposited coating for on-line in-tube solid phase microextraction of aldehydes in human exhaled breath condensate. *Journal of Chromatography A*, *1395*, 23–31.

Liang, T., Gao, L., Qin, D., & Chen, L. (**2019**). Determination of sulfonylurea herbicides in grain samples by matrix solid phase dispersion with mesoporous structured molecularly imprinted polymer. *Food Analytical Methods*, *12*(9), 1938–1948.

Lin, S., Gan, N., Cao, Y., Chen, Y., & Jiang, Q. (**2016**). Selective dispersive solid phase extraction chromatography tandem mass spectrometry based on aptamer functionalized UiO-66-NH_2 for determination of polychlorinated biphenyls. *Journal of Chromatography A*, *1446*, 34–40.

Liu, Q., Li, Z., Wei, L., Chen, X., Xu, Y., & Zhao, J. (**2022**). Fast dispersive liquid–liquid microextraction of pesticides in water based on a thermo switchable deep eutectic solvent. *Environmental Chemistry Letters*, *20*(4), 2271–2276.

Liu, T., Cao, P., Geng, J., Li, J., Wang, M., Wanga, M., Li, X., & Yin, D. (**2014**). Determination of triazine herbicides in milk by cloud point extraction and high performance liquid chromatography. *Food Chemistry*, *142*, 358–364.

Lucas, V., de, P. F., Lorena, M. G. A., L´azaro, C. S., Gevany, P. de P., & Flaviano, O. S. (**2021**). Determination of DDT in honey samples by liquid–liquid extraction with low temperature purification (LLE–LTP) combined to HPLC–DAD. *Analytical Methods*, *13*, 1955–1964.

Ma, L., Wang, Y., Li, H., Peng, F., Qiu, B., Ma, L., Wang, Y., Li, H., Peng, F., Qiu, B., & Yang, Z. (**2020**). Development of QuEChERS–DLLME method for determination of neonicotinoid pesticide residues in grains by liquid chromatography–tandem mass spectrometry. *Food Chemistry*, *331*, 127190.

Mahpishanian, S., & Sereshti, H. (**2014**). Graphene oxide based dispersive micro solid phase extraction for separation and preconcentration of nicotine from biological and environmental water samples followed by gas chromatography–flame ionization detection. *Talanta*, *130*, 71–77.

Mahpishanian, S., & Sereshti, H. (**2016**). Three dimensional graphene aerogel supported iron oxide nanoparticles as an efficient adsorbent for magnetic solid phase extraction of organophosphorus pesticide residues in fruit juices followed by gas chromatographic determination. *Journal of Chromatography A*, *1443*, 43–53.

Majlesi, M., & Massoudinejad, M. (**2016**). Simultaneous separation and preconcentration of phosalone and chlorpyrifos in fresh vegetables. *Analytical Methods*, *8*, 3795–3801.

Mao, X. (**2020**). Analysis of organophosphorus and pyrethroid pesticides in organic and conventional vegetables using QuEChERS combined with dispersive liquid–liquid microextraction based on the solidification of floating organic droplet. *Food Analytical Methods*, *309*, 125755.

Marta, P. B., Isabel, G., Natalia, C., Pilar, V., Pilar, H., Pilar, F., & José, F. (**2017**). Combination of solvent extractants for dispersive liquid–liquid microextraction of fungicides from water and fruit samples by liquid chromatography with tandem mass spectrometry. *Food Chemistry*, *233*, 69–76.

Mayara, J., Almeida, F. De, Maria, E., & Silva, F. (**2020**). Salting out assisted liquid–liquid extraction method combined with GC–MS for the determination of topiramate in aqueous solutions : development and application of the methodology. *Separation Science and Technology*, *55*(13), 2303-2312.

Mengistie, B. T., Mol, A. P. J., Oosterveer, P., & Simane, B. **(2015)**. Information, motivation and resources: the missing elements in agricultural pesticide policy implementation in Ethiopia. *International Journal of Agricultural Sustainability*, *13*(3), 240–256.

Megersa, N. **(2015**). Hollow fiber liquid phase microextraction for trace enrichment of the residues of atrazine and its major degradation products from environmental water and human urine samples. *Analytical Methods*, *7*(23), 9940–9948.

Mehlhorn, P., Humphries, M., Gensel, J., Buah-Kwofie, A., Tyohemba, R. L., & Haberzettl, T. (**2022**). Organochlorine pesticide contamination in sediments from Richards Bay, South Africa: spatial trends and ecotoxicological risks. *Environmental Science and Pollution Research*, *30*(1), 2247–2259.

Mekonen, S., Ambelu, A., Negassa, B., & Spanoghe, P. (**2017**). Exposure to DDT and its metabolites from khat (Catha edulis) chewing: Consumers risk assessment from southwestern Ethiopia. *Regulatory Toxicology and Pharmacology*, *87*, 64–70.

Mekonen, S., Ambelu, A., & Spanoghe, P. (**2014**). Pesticide residue evaluation in major staple food items of Ethiopia using the QuEChERS method: A case study from the jimma zone. *Environmental Toxicology and Chemistry*, *33*(6), 1294–1302.

Merdassa, Y., Liu, J. F., & Megersa, N. (**2013**). Development of a one step microwave assisted extraction method for simultaneous determination of organophosphorus pesticides and fungicides in soils by gas chromatography–mass spectrometry. *Talanta*, *114*, 227–234.

Merdassa, Y., Liu, J. F., & Megersa, N. (**2015a**). Development and validation of one step ultrasound assisted extraction for simultaneous determination of multiclass fungicides in soils. *Journal of AOAC International*, *98*(1), 192–200.

Merdassa, Y., Liu, J. F., Megersa, N., & Tessema, M. **(2015b**). An efficient and fast microwave-assisted extraction method developed for the simultaneous determination of 18 organochlorine pesticides in sediment. *International Journal of Environmental Analytical Chemistry*, *95*(3), 225–239.

Mezcua, M., Repetti, M. R., Agüera, A., Ferrer, C., García-Reyes, J. F., & Fernández-Alba, A. R. (**2007**). Determination of pesticides in milk based infant formulas by pressurized liquid extraction followed by gas chromatography tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, *389*(6), 1833–1840.

Mirnaghi, F. S., Goryński, K., Rodriguez-Lafuente, A., Boyaci, E., Bojko, B., & Pawliszyn, J. (**2013**). Microextraction versus exhaustive extraction approaches for simultaneous analysis of compounds in wide range of polarity. *Journal of Chromatography A*, *1316*, 37–43.

Mnyandu, H. M., & Mahlambi, P. N. (**2021**). 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*, 129818.

Mohammadnia, M., Heydari, R., & Sohrabi, M. R. (**2020**). Determination of 2,4-Dichlorophenoxyacetic acid in food and water samples using a modified graphene oxide sorbent and high performance liquid chromatography. *Journal of Environmental Science and Health, Part B*, *55*(4), 293–300.

Mohd, N. I., Zain, N. N. M., Raoov, M., & Mohamad, S. (**2018**). Determination of carcinogenic herbicides in milk samples using green non ionic silicone surfactant of cloud point extraction and spectrophotometry. *Royal Society Open Science*, *5*(4), 171500.

Monireh, M., Mohamadreza, M., Fateh, H., & Nazir, F. (**2016**). Simultaneous separation and preconcentration of phosalone and chlorpyrifos in fresh vegetables using ultrasound assisted dispersive liquid–liquid microextraction and high performance liquid chromatography. *Analytical Methods*, *8*, 3795–3801.

Moradi Shahrebabak, S., Saber-Tehrani, M., Faraji, M., Shabanian, M., & Aberoomand-Azar, P. (**2019**). Simultaneous magnetic solid phase extraction of acidic and basic pesticides using triazine based polymeric network modified magnetic nanoparticles/graphene oxide nanocomposite in water and food samples. *Microchemical Journal*, *146*, 630–639.

Moreno-gonzález, Ana M, D., & García-campaña. (**2017**). Salting out assisted liquid–liquid extraction coupled to ultra high performance liquid chromatography–tandem mass spectrometry for the determination of tetracycline residues in infant foods. *Food Chemistry*, *221*, 1763–1769.

Moreno-gonzález, D., Gámiz-gracia, L., Bosque-sendra, J. M., & García-campa, A. M. (**2012**). Dispersive liquid–liquid microextraction using a low density extraction solvent for the determination of 17 N-methylcarbamates by micellar electrokinetic chromatography electrospray–mass spectrometry employing a volatile surfactant. *Journal of Chromatography A*, *1247*, 26–34.

Muckoya, V. A., Nomngongo, P. N., & Ngila, J. C. (**2020**). Determination of organophosphorus pesticides in wastewater samples using vortex assisted dispersive liquid–liquid microextraction with liquid chromatography–mass spectrometry. *International Journal of Environmental Science and Technology*, *17*(4), 2325–2336.

Nakamura, K., Otake, T., & Hanari, N. (**2019**). Evaluation of pressurized liquid extraction for the determination of neonicotinoid pesticides in green onion. *Journal of Environmental Science and Health, Part B, 54* (8) 640–646.

Nakao, T., & Banba, S. (**2015**). Minireview: Mode of action of meta-diamide insecticides. *Pesticide Biochemistry and Physiology*, *121*, 39–46.

Nasiri, M., Ahmadzadeh, H., & Amiri, A. (**2020**). Sample preparation and extraction methods for pesticides in aquatic environments: A review. *Trends in Analytical Chemistry*, *123*, 115772.

Naureen, B., Haseeb, A. S. M. A., Basirun, W. J., & Muhamad, F. (**2020**). Assessing the impact of industrial waste on environment and mitigation strategies: a comprehensive review. *Journal of Hazardous Materials*, *398,* 123019.

Negatu, B., Kromhout, H., Mekonnen, Y., & Vermeulen, R. (**2016**). Use of chemical pesticides in Ethiopia: A cross-sectional comparative study on knowledge, attitude and practice of farmers and farm workers in three farming systems. *Annals of Occupational Hygiene*, *60*(5), 551–566.

Negussie, B. T., Dube, S., & Nindi, M. M. (**2021**). Multiclass pesticide residue analysis in fruit and vegetable samples by combining acetone based salting out assisted extraction with dispersive liquid–liquid microextraction. *Journal of Chemistry*, *2021*, 1–12.

Nisha, S., Pragati, T., & Manohar, G. C. (**2021**). Determination of eight endocrine disruptor pesticides in bovine milk at trace levels by dispersive liquid–liquid microextraction followed by GC–MS determination. *Journal of Separation Science*, *44*(15), 2982–2995.

Niu, Z., Yu, C., He, X., Zhang, J., & Wen, Y. (**2017**). Salting out assisted liquid–liquid extraction combined with gas chromatography–mass spectrometry for the determination of pyrethroid insecticides in high salinity and biological samples. *Journal of Pharmaceutical and Biomedical Analysis*, *143*, 222–227.

Nodeh, H. R., Wan Ibrahim, W. A., Sanagi, M. M., & Aboul-Enein, H. Y. (**2016**). Magnetic graphene based cyanopropyltriethoxysilane as an adsorbent for simultaneous determination of polar and non polar organophosphorus pesticides in cow's milk. *RSC Advances*, *6*(30), 24853–24864.

Nováková, L., & Vlčková, H. (**2009**). A review of current trends and advances in modern bioanalytical methods: Chromatography and sample preparation. *Analytica Chimica Acta*, *656*(1–2), 8–35.

Nsibande, S. A., & Forbes, P. B. C. (**2016**). Fluorescence detection of pesticides using quantum dot materials – A review. *Analytica Chimica Acta*, *945*, 9–22.

Ntombela, S. C., & Mahlambi, P. N. (**2019**). Method development and application for triazine herbicides analysis in water, soil and sediment samples from KwaZulu-Natal. *Journal of Environmental Science and Health - Part B*, *54*(7), 569–579.

Oesterlund, A. H., Thomsen, J. F., Sekimpi, D. K., Maziina, J., Racheal, A., & Jørs, E. (**2019**). Pesticide knowledge, practice and attitude and how it affects the health of small scale farmers in Uganda: A cross sectional study. *African Health Sciences*, *14*(2), 420–433.

Olisah, C., Adeniji, A. O., Okoh, O. O., & Okoh, A. I. (**2019**). Occurrence and risk evaluation of organochlorine contaminants in surface water along the course of Swartkops and Sundays River Estuaries, Eastern Cape Province, South Africa. *Environmental Geochemistry and Health*, *41*(6), 2777–2801.

Pasupuleti, R. R., Gurrani, S., Tsai, P., & Ponnusamy, V. K. (**2020**). Novel salt assisted liquid–liquid microextraction technique for environmental, food, and biological samples analysis applications : A review. *Current Analytical Chemistry*, *17*, 1–11.

Pei, M., Zhu, X., & Huang, X. (**2018**). Mixed functional monomers based monolithic adsorbent for the effective extraction of sulfonylurea herbicides in water and soil samples. *Journal of Chromatography A*, *1531*, 13–21.

Peris, A., Barbieri, M. V., Postigo, C., Rambla-Alegre, M., López de Alda, M., & Eljarrat, E. (**2022**). Pesticides in sediments of the Ebro River Delta cultivated area (NE Spain): Occurrence and risk assessment for aquatic organisms. *Environmental Pollution*, *305*, 119239.

Perreau, F., Bados, P., Kerhoas, L., Nélieu, S., & Einhorn, J. (**2007**). Trace analysis of sulfonylurea herbicides and their metabolites in water using a combination of off-line or on-line solid phase extraction and liquid chromatography–tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, *388*(5–6), 1265–1273.

Pirsaheb, M., Fattahi, N., Pourhaghighat, S., & Shamsipur, M. (**2015**). Simultaneous determination of imidacloprid and diazinon in apple and pear samples using sonication and dispersive liquid–liquid microextraction. *LWT - Food Science and Technology*, *60*(1), 825–831.

Pirsaheb, M., Fattahi, N., Rahimi, R., Sharafi, K., & Ghaffari, H. R. (**2017**). Evaluation of abamectin , diazinon and chlorpyrifos pesticide residues in apple product of Mahabad region gardens : Iran in 2014. *Food Chemistry*, *231*, 148–155.

Rai, S., Singh, A. K., Srivastava, A., & Yadav, S. (**2016**). Comparative evaluation of QuEChERS method coupled to DLLME extraction for the analysis of multiresidue pesticides in vegetables and fruits by gas chromatography–mass spectrometry. *Food Analytical Methods*, *9*, 2656–2669.

Ramos, R., Carvalho, R., Dela, M., Rodriguez, V., Franco, E. S., Beltrame, F., Pereira, A. L., Santos, V. S., Araujo, W., & Rocha, B. A. (**2020**). DLLME–SFO–GC–MS procedure for the determination of 10 organochlorine pesticides in water and remediation using magnetite nanoparticles. *Environmental Science and Pollution Research*, *27*, 45336–45348.

Rashidipour, M., Heydari, R., Maleki, A., Mohammadi, E., & Davari, B. (**2018**). Salt assisted liquid–liquid extraction coupled with reversed phase dispersive liquid–liquid microextraction for sensitive HPLC determination of paraquat in environmental and food samples. *Journal of Food Measurement and Characterization*, *13*, 269–276.

Rawat, K., Srivastava, A., Tandon, S., & Pal, G. (**2023**). Method validation for simultaneous determination of four neonicotinoids in vegetables by liquid chromatography. *Analytical Sciences*, *39*, 431–439.

Razmi, H., & Jabbari, M. (**2015**). Development of graphene carbon nanotube coated magnetic nanocomposite as an efficient sorbent for HPLC determination of organophosphorus pesticides in environmental water samples. *International Journal of Environmental Analytical Chemistry*, *95*(14), 1353–1369.

Rejczak, T., & Tuzimski, T. (**2017**). QuEChERS based extraction with dispersive solid phase extraction cleanup using PSA and ZrO_2 based sorbents for determination of pesticides in bovine milk samples by HPLC-DAD. *Food Chemistry*, *217*, 225–233.

Rezaee, M., Assadi, Y., Milani Hosseini, M. R., Aghaee, E., Ahmadi, F., & Berijani, S. (**2006**). Determination of organic compounds in water using dispersive liquid–liquid microextraction. *Journal of Chromatography A*, *1116*(1–2), 1–9.

Riahi-Zanjani, B., Balali-Mood, M., Asoodeh, A., Es'haghi, Z., & Ghorani-Azam, A. (**2018**). Developing a new sensitive solid phase microextraction fiber based on carbon nanotubes for preconcentration of morphine. *Applied Nanoscience*, *8*(8), 2047–2056.

Rodrigues, F. de M., Mesquita, P. R. R., de Oliveira, L. S., de Oliveira, F. S., Menezes Filho, A., de P. Pereira, P. A., & de Andrade, J. B. (**2011**). Development of a headspace solid phase microextraction/gas chromatography–mass spectrometry method for determination of organophosphorus pesticide residues in cow milk. *Microchemical Journal*, *98*(1), 56–61.

Roldán-Pijuán, M., Lucena, R., Cárdenas, S., Valcárcel, M., Kabir, A., & Furton, K. G. (**2015**). Stir fabric phase sorptive extraction for the determination of triazine herbicides in environmental waters by liquid chromatography. *Journal of Chromatography A*, *1376*, 35–45.

Rouhollah, H., & Rezvan, F. (**2021**). Determination of 2,4-dichlorophenoxyacetic acid in water and edible seeds samples using salt assisted liquid–liquid extraction coupled with high performance liquid chromatography. *Food Analytical Methods*, *14*, 561–567.

Salemi, A., Rasoolzadeh, R., Nejad, M. M., & Vosough, M. (**2013**). Ultrasonic assisted headspace single drop microextraction and gas chromatography with nitrogen-phosphorus detector for determination of organophosphorus pesticides in soil. *Analytica Chimica Acta*, *769*, 121–126.

Salis, A., & Ninham, B. W. (**2014**). Models and mechanisms of Hofmeister effects in electrolyte solutions, and colloid and protein systems revisited. *Chemical Society Reviews*, *43*(21), 7358–7377.

Samsidar, A., & Shaarani, S. (**2018**). A review of extraction, analytical and advanced methods for determination of pesticides in environment and foodstuffs. *Trends in Food Science & Technology*, *71*, 188–201.

Sanchez-Ortega, A., Unceta, N., Gómez-Caballero, A., Sampedro, M. C., Akesolo, U., Goicolea, M. A., & Barrio, R. J. (**2009**). Sensitive determination of triazines in underground waters using stir bar sorptive extraction directly coupled to automated thermal desorption and gas chromatography–mass spectrometry. *Analytica Chimica Acta*, *641*(1–2), 110–116.

See, H. H., Marsin Sanagi, M., Ibrahim, W. A. W., & Naim, A. A. (**2010**). Determination of triazine herbicides using membrane protected carbon nanotubes solid phase membrane tip extraction prior to micro liquid chromatography. *Journal of Chromatography A*, *1217*(11), 1767–1772.

Sereshti, H., Khosraviani, M., Samadi, S., & Amini-Fazl, M. S. (**2014**). Simultaneous determination of theophylline, theobromine and caffeine in different tea beverages by graphene oxide based ultrasonic assisted dispersive micro solid phase extraction combined with HPLC-UV. *RSC Advances*, *4*(87), 47114–47120.

Shamsipur, M., Yazdanfar, N., & Ghambarian, M. (**2016**). Combination of solid phase extraction with dispersive liquid–liquid microextraction followed by GC–MS for determination of pesticide residues from water, milk, honey and fruit juice. *Food Chemistry*, *204*, 289–297.

Sharafi, K., Fattahi, N., Mahvi, A. H., Pirsaheb, M., Azizzadeh, N., & Noori, M. (**2015**). Trace analysis of some organophosphorus pesticides in rice samples using ultrasound assisted dispersive liquid–liquid microextraction and high performance liquid chromatography. *Journal of Separation Science*, *38*(6), 1010–1016.

Sherin, F. H., Inas A. A., Alaa, B., & Fotouh, R. M. (**2022**). Homogeneous liquid–liquid extraction as an alternative sample preparation technique for biomedical analysis. *Journal of Separation Science*, *45*(1), 185–208.

Shi, Z., Zhang, S., Huai, Q., Xu, D., & Zhang, H. (**2017**). Methylamine modified graphene based solid phase extraction combined with UPLC–MS/MS for the analysis of neonicotinoid insecticides in sunflower seeds. *Talanta*, *162*, 300–308.

Song, W., Zhang, Y., Li, G., Chen, H., Wang, H., Zhao, Q., He, D., Zhao, C., & Ding, L. (**2014**). A fast, simple and green method for the extraction of carbamate pesticides from rice by microwave assisted steam extraction coupled with solid phase extraction. *Food Chemistry*, *143*, 192–198.

Souza-Silva, É. A., Gionfriddo, E., & Pawliszyn, J. (**2015**). A critical review of the state of the art of solid phase microextraction of complex matrices II. Food analysis. *Trends in Analytical Chemistry*, *71*, 236–248.

Sparks, T. C., & Nauen, R. (**2015**). IRAC: Mode of action classification and insecticide resistance management. *Pesticide Biochemistry and Physiology*, *121*, 122–128.

Stocka, J., Tankiewicz, M., Biziuk, M., & Namieśnik, J. (**2011**). Green aspects of techniques for the determination of currently used pesticides in environmental samples. *International Journal of Molecular Sciences*, *12*(11), 7785–7805.

Sun, P., Gao, Y. L., Xu, C., & Lian, Y. F. (**2018**). Determination of six organophosphorus pesticides in water samples by three dimensional graphene areogel based solid phase extraction combined with gas chromatography–mass spectrometry. *RSC Advances*, *8*(19), 10277–10283.

Sun, T., Jin, Y., Yang, J., Li, L., Shi, X., & Li, X. (**2015**). Dispersive solid phase extraction of organophosphorus pesticides from apple, cucumber and water samples using reduced graphene oxide coated with ZnO nanocomposites as a sorbent. *Analytical Methods*, *7*(15), 6095–6102.

Tankiewicz, M., Fenik, J., & Biziuk, M. (**2010**). Determination of organophosphorus and organonitrogen pesticides in water samples. *Trends in Analytical Chemistry*, *29*(9), 1050–1063.

Tankiewicz, M., Fenik, J., & Biziuk, M. (**2011**). Solventless and solvent minimized sample preparation techniques for determining currently used pesticides in water samples: A review. *Talanta*, *86*(1), 8–22.

Tejada-casado, C., Lara, F. J., García-campa, A. M., & Olmo-iruela, M. (**2018**). Ultra high performance liquid chromatography with fluorescence detection following salting out assisted liquid–liquid extraction for the analysis of benzimidazole residues in farm fish samples. *Journal of Chromatography A*, *1543*, 58-66.

Teju, E., Tadesse, B., & Megersa, N. (**2021**). Salting out assisted liquid–liquid extraction for the preconcentration and quantitative determination of 8 herbicide residues simultaneously in different water samples with high performance liquid chromatography. *Separation Science and Technology*, 56(4), 719-729.

Teklu, B. M., Adriaanse, P. I., & Van den Brink, P. J. (**2016**). Monitoring and risk assessment of pesticides in irrigation systems in Debra Zeit, Ethiopia. *Chemosphere*, *161*, 280–291.

Tessema, R. A., Nagy, K., & Ádám, B. (**2021**). Pesticide use, perceived health risks and management in Ethiopia and in Hungary: A Comparative analysis. *International Journal of Environmental Research and Public Health*, *18*(19), 10431.

Tian, H., Bai, X., & Xu, J. (**2017**). Simultaneous determination of simazine, cyanazine, and atrazine in honey samples by dispersive liquid–liquid microextraction combined with high performance liquid chromatography. *Journal of Separation Science*, *40*(19), 3882–3888.

Tighrine, A., Amir, Y., Alfaro, P., Mamou, M., & Nerín, C. (**2019**). Simultaneous extraction and analysis of preservatives and artificial sweeteners in juices by salting out liquid–liquid extraction method prior to ultra high performance liquid chromatography. *Food Chemistry*, *277*, 586–594.

Timofeeva, I., Shishov, A., Kanashina, D., Dzema, D., & Bulatov, A. (**2017**). On-line in-syringe sugaring out liquid–liquid extraction coupled with HPLC–MS/MS for the determination of pesticides in fruit and berry juices. *Talanta*, *167*, 761–767.

Tiryaki, O., & Temur, C. (**2010**). The fate of pesticide in the environment. *Journal of Biodiversity and Environmental Sciences*, *4*(10), 29–38.

Tolcha, T., & Megersa, N. (**2018**). High density solvent based dispersive liquid–liquid microextraction technique for simultaneous and selective extraction of multiclass pesticide residues in water and sugarcane juice samples. *American Journal of Analytical Chemistry*, *9*, 224–244.

Tolcha, T., Meredasa, Y., & Megersa, N. (**2013**). Low density extraction solvent based solvent terminated dispersive liquid–liquid microextraction for quantitative determination of ionizable pesticides in environmental waters. *Journal of Separation Science, 36*, 1119–1127.

Tolcha, T., & Megersa, N. (**2021**). SALLE combined with LD–DLLME for pesticides analysis in sugar and soil samples. *Bulletin of the Chemical Society of Ethiopia*, *35*(1), 1–16.

Tolcha, T., Gemechu, T., Al-Hamimi, S., Megersa, N., & Turner, C. (**2020**). High density supercritical carbon dioxide for the extraction of pesticide residues in onion with multivariate response surface methodology. *Molecules, 25*(1012), 1-11.

Tolcha, T., Gemechu, T., Al-Hamimi, S., Megersa, N., & Turner, C. **(2021)**. Multivariate optimization of a combined static and dynamic supercritical fluid extraction method for trace analysis of pesticides pollutants in organic honey. *Journal of Separation Science, 44*, 1716–1726.

Tudi, M., Ruan, H. D., Wang, L., Lyu, J., Sadler, R., Connell, D., Chu, C., & Phung, D. T. (**2021**). Agriculture development, pesticide application and its impact on the environment. *International Journal of Environmental Research and Public Health*, *18*(3), 1–24.

Tursen, J., Yang, T., Bai, L., Li, D., & Tan, R. (**2021**). Determination of imidacloprid and acetamiprid in bottled juice by a new DLLME–HPLC. *Environmental Science and Pollution Research*, *28*(36), 50867–50877.

Valmorbida, I., Hohenstein, J. D., Coates, B. S., Bevilaqua, J. G., Menger, J., Hodgson, E. W., Koch, R. L., & O'Neal, M. E. (**2022**). Association of voltage gated sodium channel mutations with field evolved pyrethroid resistant phenotypes in soybean aphid and genetic markers for their detection. *Scientific Reports*, *12*(1), 12020.

Viñas, P., Martínez-Castillo, N., Campillo, N., & Hernández-Córdoba, M. (**2010**). Liquid–liquid microextraction methods based on ultrasound assisted emulsification and single drop coupled to gas chromatography–mass spectrometry for determining strobilurin and oxazole fungicides in juices and fruits. *Journal of Chromatography A*, *1217*(42), 6569–6577.

Wang, K., Xie, X., Zhang, Y., Huang, Y., Zhou, S., Zhang, W., Lin, Y., & Fan, H. (**2018a**). Combination of microwave assisted extraction and ultrasonic assisted dispersive liquid–liquid microextraction for separation and enrichment of pyrethroids residues in Litchi fruit prior to HPLC determination. *Food Chemistry, 240*, 1233-1242.

Wang, P., Luo, M., Liu, D., Zhan, J., Liu, X., Wang, F., Zhou, Z., & Wang, P. (**2018b**). Application of a magnetic graphene nanocomposite for organophosphorus pesticide extraction in environmental water samples. *Journal of Chromatography A*, *1535*, 9–16.

Wang, X. C., Shu, B., Li, S., Yang, Z. G., & Qiu, B. (**2017**). QuEChERS followed by dispersive liquid–liquid microextraction based on solidification of floating organic droplet method for organochlorine pesticides analysis in fish. *Talanta*, *162*, 90–97.

Wang, X., Meng, X., Wu, Q., Wang, C., & Wang, Z. (**2019a**). Solid phase extraction of carbamate pesticides with porous organic polymer as adsorbent followed by high performance liquid chromatography–diode array detection. *Journal of Chromatography A*, *1600*, 9–16.

Wang, X., Jia, R., Song, Y., Wang, M., Zhao, Q., & Sun, S. (**2019b**). Determination of pesticides and their degradation products in water samples by solid phase extraction coupled with liquid chromatography–mass spectrometry. *Microchemical Journal*, *149*, 104013.

Wang, Y. L., You, L. Q., Mei, Y. W., Liu, J. P., & He, L. J. (**2016a**). Benzyl functionalized ionic liquid as new extraction solvent of dispersive liquid–liquid microextraction for enrichment of organophosphorus pesticides and aromatic compounds. *Chinese Journal of Analytical Chemistry*, *44*(6), 942–949.

Wang, Y., Miao, X., Wei, H., Liu, D., & Xia, G. (**2016b**). Dispersive liquid–liquid microextraction combined with gas chromatography–mass spectrometry for the determination of multiple pesticides in celery. *Food Analytical Methods*, *9*, 2133–2141.

Wei, S. Y., Leong, M. I., Li, Y., & Huang, S. Da. (**2011**). Development of liquid phase microextraction based on manual shaking and ultrasound assisted emulsification method for analysis of organochlorine pesticides in aqueous samples. *Journal of Chromatography A*, *1218*(51), 9142–9148.

Wen, Y., Li, J., Yang, F., Zhang, W., Li, W., & Liao, C. (**2013**). Salting out assisted liquid–liquid extraction with the aid of experimental design for determination of benzimidazole fungicides in high salinity samples by high performance liquid chromatography. *Talanta*, *106*, 119–126.

WHO. (**2019**). The WHO recommended classification of pesticides by hazard and guidelines to classification. Geneva, https://iris.who.int/bitstream/handle/10665/332193/9789240005662-eng.pdf?ua%3D1

WHO. **(2017)**. Guidelines for drinking water quality. 4th ed, incorporating First addendum, World Health Organization, Geneva, Switzerland.

Wu, Q. H., Li, Y. P., Li, C., Wu, C. X., Liu, Z. M., Hou, Y. X., & Wang, Z. (**2010**). Dispersive liquid–liquid microextraction combined with high performance liquid chromatography–DAD detection for the determination of sulfonylurea herbicides in water samples. *International Journal of Environmental Analytical Chemistry*, *90*, 891–902.

Xu, Q., Qiao, K., Yan, C., Liu, Z., Lu, R., & Zhou, W. (**2020**). Dispersive micro solid phase extraction based on a graphene/polydopamine composite for the detection of pyrethroids in water samples. *Analytical Methods*, *12*(24), 3115–3122.

Xu, S. X., Zhang, L. L., Zhang, H., Zeng, Y., & Shi, Z. (**2016**). A salt assisted graphene oxide aggregation method for the determination of dimethylamine and trimethylamine by ion chromatography with conductivity detection. *Analytical Methods*, *8*, 1828-1835.

Yan, L., Jing, Z., Lin, R., Yongxin, L., & Xiaoli, Z. (**2016**). Application of solvent demulsification dispersive liquid–liquid microextraction based on solidification of floating organic drop coupled with high perfomence liquid chromatography in determination of sulfonylurea herbicides in water and soil. *Journal of Brazilian Chemical Society*, 27(10), 1792-1799.

Yang, J. (**2017**). A novel method for the determination of trace sulfonylurea herbicides by introducing a hybrid stationary phase to common capillary. *Adsorption Science & Technology*, *35*(3–4), 372–385.

Yin, L., Hu, Q., Mondal, S., Xu, J., & Ouyang, G. (**2019**). Peanut shell derived biochar materials for effective solid phase microextraction of polycyclic aromatic hydrocarbons in environmental waters. *Talanta*, *202*, 90–95.

Yohannes, A., Tolesa, T., Merdassa, Y., & Megersa, N. (**2016**). Single drop microextraction analytical technique for simultaneous separation and trace enrichment of atrazine and its major degradation products from environmental waters followed by liquid chromatographic determination. *Journal of Analytical & Bioanalytical Techniques*, *7*(5), 1000330.

You, X., & Chen, L. (**2016**). Analysis of sulfonylurea herbicides in grain samples using molecularly imprinted polymers on the surface of magnetic carbon nanotubes by extraction coupled with HPLC. *Analytical Methods*, *8*(5), 1003–1012.

Zeiadi, S., Reza, M., Mogaddam, A., & Farajzadeh, M. A. (**2020**). Combination of dispersive solid phase extraction with lighter than water dispersive liquid–liquid microextraction for the extraction of organophosphorous pesticides from milk. *International Journal of Environmental Analytical Chemistry*, *102*(17), 5873-5886.

Zhang, H., Yuan, Y., Sun, Y., & Niu, C. (**2017**). An ionic liquid magnetic graphene composite for magnet dispersive solid phase extraction of triazine herbicides in surface water followed by high performance liquid chromatography. *Analyst*, *143*, 175–181.

Zhang, L., Yu, R., Wang, Z., Li, N., Zhang, H., & Yu, A. (**2014a**). Determination of triazine herbicides in vegetables by ionic liquid foam floatation solid phase extraction high performance liquid chromatography. Journal of Chromatography B, 953–954, 132–137.

Zhang, Y., Zhang, X., & Jiao, B. (**2014b**). Determination of ten pyrethroids in various fruit juices : Comparison of dispersive liquid–liquid microextraction sample preparation and QuEChERS method combined with dispersive liquid–liquid microextraction. *Food Chemistry*, *159*, 367–373.

Zhao, G., Song, S., Wang, C., Wu, Q., & Wang, Z. (**2011**). Determination of triazine herbicides in environmental water samples by high performance liquid chromatography using graphene coated magnetic nanoparticles as adsorbent. *Analytica Chimica Acta*, *708*, 155–159.

Zhao, L., Szakas, T., Churley, M., & Lucas, D. (**2019**). Multiclass multiresidue analysis of pesticides in edible oils by gas chromatography–tandem mass spectrometry using liquid–liquid extraction and enhanced matrix removal lipid cartridge cleanup. *Journal of Chromatography A*, *1584*, 1–12.

Zhao, X., Zhou, Y., Kong, W., Gong, B., Chen, D., Wei, J., & Yang, M. (**2016**). Multiresidue analysis of 26 organochlorine pesticides in Alpinia oxyphylla by GC–ECD after solid phase extraction and acid cleanup. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *1017–1018*, 211–220.

Zhou, Q., & Fang, Z. (**2015a**). Graphene modified TiO_2 nanotube arrays as an adsorbent in micro solid phase extraction for determination of carbamate pesticides in water samples. *Analytica Chimica Acta*, *869*, 43–49.

Zhou, Q., & Fang, Z. **(2015b**). Use of TiO_2 nanotube arrays as the adsorbents for preconcentration of triazine herbicides in environmental water samples prior to determination using high performance liquid chromatography. *Analytical Methods*, *7*(7), 3277–3282.

Zhu, L., Huey Ee, K., Zhao, L., & Lee, H. K. (**2002**). Analysis of phenoxy herbicides in bovine milk by means of liquid–liquid–liquid microextraction with a hollow fiber membrane. *Journal of Chromatography A*, *963*(1–2), 335–343.

Zygler, A., Wasik, A., & Namieśnik, J. (**2010**). Retention behaviour of some high intensity sweeteners on different SPE sorbents. *Talanta*, *82*(5), 1742–1748.

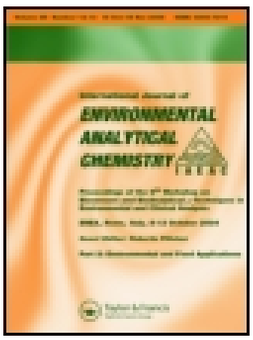
Paper – I

(Published)

Green analytical method based on salt assisted graphene oxide dispersive solid phase of symmetrical triazine herbicides in environmental water samples for liquid chromatographic determination

Habtamu Bekele and Negussie Megersa

International journal of environmental analytical chemistry, 2023, 1-17.
https://doi.org/10.1080/03067319.2022.216137



International Journal of Environmental Analytical Chemistry

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/geac20

Green analytical method based on salt assisted graphene oxide dispersive solid phase extraction of symmetrical triazine herbicides in environmental water samples for liquid chromatographic determination

Habtamu Bekele & Negussie Megersa

To cite this article: Habtamu Bekele & Negussie Megersa (2023): Green analytical method based on salt assisted graphene oxide dispersive solid phase extraction of symmetrical triazine herbicides in environmental water samples for liquid chromatographic determination, International Journal of Environmental Analytical Chemistry, DOI: 10.1080/03067319.2022.2161377

To link to this article: https://doi.org/10.1080/03067319.2022.2161377

Published online: 03 Jan 2023.

INTERNATIONAL JOURNAL OF ENVIRONMENTAL ANALYTICAL CHEMISTRY
https://doi.org/10.1080/03067319.2022.2161377

Green analytical method based on salt assisted graphene oxide dispersive solid phase extraction of symmetrical triazine herbicides in environmental water samples for liquid chromatographic determination

Habtamu Bekele and Negussie Megersa

Department of Chemistry, College of Natural and Computational Sciences, Addis Ababa University, Addis Ababa, Ethiopia

ABSTRACT

Graphene oxide (GO) was used as sorbent in dispersive solid phase extraction (DSPE) to enrich and extract some triazine herbicides from environmental water samples. It can form dispersed colloids in aqueous phase and provides rich functional groups for the formation of hydrogen bonding or π–π electrostatic interaction with benzene ring organic compounds containing oxygen and nitrogen functional groups. GO was synthesised by modified Hummers method and characterised with Fourier transform infrared (FT-IR) spectroscopy. As complete collection of GO after adsorption process is difficult task, in this established salt-assisted (SA)-GO-DSPE method, it was found to be aggregated and centrifuged in the presence of NaCl. The optimum value for main parameters that influenced extraction efficiency were investigated. Under the optimised conditions, low limits of detection (LODs) (0.12–0.80 ng mL $^{-1}$), limits of quantification (LOQ) (0.4–2.68 ng mL $^{-1}$), and good extraction recoveries (69.06–95.53%) were obtained for simultaneous extraction of simazine (SIZ), atrazine (ATZ), propazine (PRZ)) and prometryn (PRN). The relative standard deviations (RSD) of intraday (n = 6) and interday (n = 9) precisions were 2.54 to 5.84% and 4.38 to 8.56%, respectively. At the end, the applicability of the method was evaluated and target analytes were not detected in real samples. The proposed method is environment friendly and promising for trace analysis of pesticides including the target herbicides in environmental water samples.

ARTICLE HISTORY
Received 16 October 2022
Accepted 15 December 2022

KEYWORDS
Trace enrichment; triazine herbicides; environmental water; salt assisted graphene oxide; green analytical method

1. Introduction

Pesticides are used as one of the most preferred choices of agriculture and health sectors because of their rapid actions on eradicating disease causing organisms, weeds, control insects, and other pests. Among these chemicals, triazine herbicides are frequently used agrichemicals around the world because of their properties of inhibiting photosynthetic reactions to help control the growth of grasses and broadleaf weeds [1,2]. However, the use of triazines has effects on the treated plants itself and the environmental compartments, such as environmental water and soil. Thus, the exposure to these compounds and

CONTACT Negussie Megersa negussie.megersa@gmail.com

their degradation products have potential acute health risks on humans such as eye irritation, dermal problems, headache, and nausea as well as chronic health effects including birth defect, cancer, the disruption of endocrine system and interruption of hormone functions [3]. To overcome the risks from these trace level residues in the environment, legislative authorities including the United States Environmental Protection Agency (US EPA) and the European Union (EU) have set the maximum residue limits (MRLs) of triazine herbicides in drinking water, and accordingly 0.5 μg L^{-1} for the total amount of triazines and 0.1 μg L^{-1} for individual triazine compounds [4]. As a result of these restrictions, there is a great demand for development of selective, simple, low cost, and environmental friendly 'green' sample preparation methods that can simultaneously preconcentrate and extract trace levels of the target analytes in water samples.

A wide variety of conventional, novel microextraction and miniaturised extraction techniques, such as solid phase extraction (SPE) [3], solid phase micro extraction (SPME) [5], supported liquid membrane extraction (SLME) [6], cloud point extraction (CPE) [7], dispersive liquid-liquid microextraction (DLLME) [8], liquid-liquid extraction (LLE) [9] and magnetic solid phase extraction (MSPE) [10], were employed for the extraction of triazine herbicide residues in food and environmental water samples. However, some of these sample preparation methods require expensive equipment's [5], are time consuming [11,12], use costly organic solvent, and requiring large sample volumes [9]. SPE is one of the most widely applied pretreatment techniques because of its high recovery, reproducibility, and simple operation [13]. In the SPE procedure, adsorbents play an important roles in achieving high analyte extraction efficiency, as the extraction takes place by the adsorption of the target compound on the surface of adsorbents [14]. So the exploration for a simple, cost effective and efficient adsorption capacity sorbent for SPE is mandatory in order to obtain a satisfactory recovery.

Graphene is a chemical substance (carbon nano-material) that contains a single-atom thick sheet of honeycomb-shaped carbon lattice, possessing a high specific surface area, which makes it extremely good adsorption ability and becoming one of a successful adsorbent of organic compounds [12,15,16]. A unique advantage of graphene over other carbon-based nanomaterials is because of the upper and lower sides of its planes which are accessible for adsorption [17]. However, pure graphene sheets tend to form unrecoverable agglomerates or restore to form graphite through Van der Waals interactions and π-stacking if the sheets are not detached from each other. Therefore, chemical modification of graphene is the most reliable means to synthesise graphene oxide (GO) which is also important in the preparation of other graphene based sorbent materials [18]. GO appears to be an ultimate sorbent in SPE because of as much quantities of oxygen atoms on its surface obtainable from hydroxyl, epoxy, and carboxyl groups. Because of these functional groups on it, GO have good affinity towards organic compounds containing benzene ring, oxygen and nitrogen functional groups through the formation of a π–π electrostatic interaction, hydrogen bonding and strong π-π stacking interaction [13,19,20]

Nowadays, graphene-based materials have been used as adsorbents for the extraction and preconcentration of pesticides such as carbamate [21,22], organophosphorus [23,24], organochlorine [20], triazine [12,16] and neonicotinoid [10]. Most of the cited literature and others use magnetised graphene-based nano materials and composite of GO. However, the synthesis of magnetised graphene-based nanomaterial adsorbents is costly and time consuming, and there have been issues when these composites have been

utilised as adsorbents. The adsorption capacity decreases as the amount of Fe_3O_4 increases because too many magnetic nanoparticles may cover the graphene sheets, necessitating the laborious and time-consuming process of optimising the amount of Fe_3O_4 nanoparticles [12]. The interaction of Fe_3O_4 nanoparticles and GO layer is only by electrostatic or physical adsorption which may be the reason for its leaching out from the GO sheets [25]. Hence, it is necessary to develop a cost effective, efficient, simple and time-saving pattern of SPE procedure using GO adsorbent for enrichment of trace level pesticides in environmental water samples.

Dispersive solid phase extraction (DSPE) is a relatively simple and fast sample preparation technique, which was developed by the United States Department of Agriculture [26]. DSPE could avoid complex process conditioning and washing, and back pressure problem often encountered in common SPE. GO have been used as the adsorption materials for DSPE. GO nanosheets are negatively charged in pH range 2–10 [27], which is consistent with the expected result that the ionisation of functional groups exist on its surface. These highly negative charges at this pH range resulted in well-dispersed GO through the strong electrostatic repulsion in water samples; but leads to difficult in collection after we use it as sorbent in DSPE. Also due to the low density of pristine GO, it is tedious work for separation in centrifugation and the high pressure in filtration. Therefore, NaCl was used to neutralise the excessive negative charges and decrease the electrostatic repulsion to carry out the purpose of GO aggregation [28].

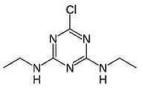
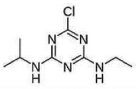
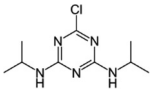
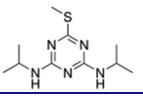
As a new mode of SPE, salt-assisted (SA)-GO-DSPE is performed with the assistance of adding NaCl salt for the purpose of GO aggregation. This mode was used for efficient extraction of triphenylmethane dyes (Malachite green and crystal violet) [28] and heavy metals (Pb, Cd, Bi, and Sb) [27] from environmental water samples and satisfactory results were obtained. To the best of our knowledge, this is the first time to use SA-GO-DSPE method coupled with HPLC-DAD for simultaneous enrichment, extraction and analysis of symmetrical triazines (*s*-triazines) such as simazine (SIZ), atrazine (ATZ), propazine (PRZ) and prometryn (PRN) herbicides from environmental water samples. Therefore, the purpose of the present work was to develop a sensitive, simple, cost effective and environmentally friendly analytical method based on SA-GO-DSPE coupled with HPLC-DAD for simultaneous extraction and analysis of four target analytes of triazine pesticides that are extensively used in agricultural farms. The developed method was then applied for tap water, spring water, river water and underground water samples.

2. Experimental

2.1. Chemicals and reagents

The target pesticide standards; viz., three chloro-*s*-triaines including atrazine (ATZ), simazine (SIZ), and propazine (PRZ), and one methylthio-*s*-triazine, i.e. prometryn (PRN) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Relevant physicochemical properties and chemical structure of the standards are provided in Table 1. Acetonitrile (ACN), used as mobile phase was of HPLC grade, and acetone were purchased from Sigma Aldrich (Steinheim, Germany). Methanol (MeOH) was received from Carlo Erba (Rodano, Italy). Chloroform was the product of Sigma Aldrich (Seelze, Germany). All other chemicals, used in this study, were of analytical grade reagents. The NaCl salts used as

Table 1. Physicochemical properties of target analytes of the s-triazine pesticides [6].

S.No	Name	Structure	Solubility, mg L^{-1} (22°C)	pK_a	log K_{ow}
1	Simazine(SIZ)		5	1.65	2.0
2	Atrazine (ATZ)		33	1.7	2.7
3	Propazine (PRZ)		8.6	1.7	3.95
4	Prometryn (PRN)		48	4.1	3.41

agglomeration of graphene oxide (GO) sorbent was received from Sigma Aldrich (Steinheim, Germany). Sodium hydroxide (NaOH; >99% purity) was the product of Merck Chemicals (Darmstadt, Germany) and used during solution pH adjustment. The graphite powder used for preparation of GO was obtained from Samchun Pure Chemical Co., Ltd. (Pyeongtaek, Korea). Sulfuric acid (H_2SO_4; >95% purity), sodium nitrate ($NaNO_3$), potassium permanganate ($KMnO_4$), hydrogen peroxide (H_2O_2), and hydrochloric acid (HCl) were all of the analytical grade reagents and purchased from Sigma Aldrich (St. Louis, MO, USA). Ultrapure water was obtained by purifying with double distiller, A 8000 Aquatron water Still (Bibby Scientific, Staffordshire, UK) and deioniser (EASYPure LF, Dubuque). It was filtered under vacuum through cellulose acetate filter papers (0.45 µm, Micro Science) before use. The stock standard solution of each target analyte with concentration of 100 µg/mL was prepared by weighing appropriate amount and dissolving it in methanol. Intermediate standard solutions of 10 µg/mL was obtained by diluting from the stock solution with methanol. Other working solutions of lower concentrtions were prepared from the intermediate solution, by diluting with methanol.

2.2. Instruments and equipment

Chromatographic analyses were performed using Agilent Technologies 1200 infinity series HPLC, equipped with quaternary pump, vacuum degasser, auto sampler and UV/Vis detector all purchased from Agilent Technologies (Waldbronn, Germany). Chromatographic separation was carried out on a ZORBAX ODS-C_{18} analytical column (150 × 3 mm i.d., 3.5 µm particle size) obtained from Agilent Technologies. Data acquisition and processing were accomplished with LC Chemstation software, (B.02, 01-R1). An ultrasonic heater, Dacon R, Dacon Laboratories Ltd (St. Hove, East Sussex), centrifuge, Model 800 (China, Beijing), 15 mL centrifuge tube, corning incorporated, (Corning, NY, Mexico) and XW-80A Vortex (Shanghai Jing Industrial Co., Ltd) were used during sample preparation. A pH metre, Adwa, model 1020 (Romania, Europe) was used for pH measurement. Identification of the various functional groups in prepared sorbent was performed using Spectrum 65 Fourier transform infrared (65 FT-IR) spectrometer.

2.3. Chromatographic conditions

Chromatographic separations were carried out following the isocratic mode of elution containing 35% ultrapure water (solvent A) and 65% ACN (solvent B), and was utilised throughout the analysis. Prior to the sample extract injection, the HPLC column was washed and conditioned with the mobile phase for 10 min. Analysis was performed at the flow rate of 1 mL min $^{-1}$, a column temperature of 35°C, and 20 µL injection volume. For quantification of the target analytes the detector was adjusted at 235 nm wavelength and then separation was achieved in 5 min run time followed by 1 min post run time. After sample injection, peak areas obtained were used for quantitative analyses.

2.4. Preparation of graphene oxide

The preparation of GO was carried out following a modified Hummer's method [29,30]. In a typical synthesis, 98 mL H_2SO_4 (95%) was added into a 600 mL beaker, and then cooled by immersion in an ice bath followed by stirring for 30 min. Then, 2.0 g graphite powder and 2.5 g $NaNO_3$ were added under vigorous stirring for 1 h. Then after, 12 g $KMnO_4$ was added gradually under stirring and the temperature of the mixture was kept to be below 10°C by cooling in ice bath and then the mixture was stirred at room temperature overnight. As the reaction proceeded, the mixture gradually became pasty and the colour turned into light brownish. To light brownish mixture 100 mL of H_2O was slowly added with vigorous agitation in an ice bath to keep the temperature below 80°C and then the diluted suspension was stirred at 98°C for 12 h. Then after, 45 mL of 30% H_2O_2 was added into the mixture to eliminate the excess MnO_4– and terminate the reaction. Finally, the mixture was centrifuged and the precipitate was washed with 5% HCl to remove traces of metal ions, followed by washing with double distilled water until the pH was 7. Finally, the GO obtained was kept drying overnight, at 80°C under vacuum.

2.5. Water samples

Various types of environmental water samples such as tap water, spring water, underground water, and river water were collected from Hambiso areas of the Degem district in North Shewa Zone, Oromia Regional State, Ethiopia. Geographical locations of the sampling sites are around 9°36′8″ N longitude and 38°19′30″E latitude at an elevation of 2, 9994 metres above sea level. Before analysis, each water sample was filtered through 0.45 µm micropore membrane filter and then stored in a polyvinyl chloride bottles, in a refrigerator, for a maximum of 24 h at 4°C without any further sample pretreatment.

2.6. The SA-GO-DSPE procedure

The SA-GO-DSPE procedure was carried out as follows: firstly, 1 mg of GO was added into 15 mL polyethylene tubes that contains 10 mL standard sample solutions at pH 5.0 and sonicated for 3 min; which was dispersion and extraction time. To this solution, 29 mg NaCl was added and GO was quickly aggregated and gradually deposited onto the bottom of the tubes. Then, complete phase separation was observed by centrifuging for 10 min at 4000 rpm. The supernatant was discarded, and the target analytes attached onto the GO nano-sheets were

eluted with 0.5 mL acetone after sonicated for 5 min. Subsequently, the eluate was collected, filtered through 0.45 µm micropore membrane filter and evaporated to dryness at 35°C. The resulting residues were then re-dissolved in 300 µL mixture of acetonitrile-water (5:1 v/v). For final analysis, 20 µL the resulting solution was injected into the HPLC and determination of the target analytes were achieved.

3. Results and discussion

3.1. Characterisation of GO

FT-IR spectrum of the GO (Figure 1) indicates that there are different oxygen containing functional groups on its surface. The characteristic bands associated to the C – O stretching vibration of epoxide were observed at 1053 cm^{-1}. The band around 1728 cm^{-1} corresponding to C = O stretching of carbonyl and carboxyl groups. The characteristic band deputed around 1617 cm^{-1} can be assigned to aromatic C = C stretches from unoxidised graphitic class. A broad characteristic band located around 3410 cm^{-1} may belong to the O – H stretching vibrations of the C – OH groups and that of water. In addition, the peaks due to the asymmetric CH_2 stretching of GO were appearing around 2924 cm^{-1}. These results are in agreement with works reported in the

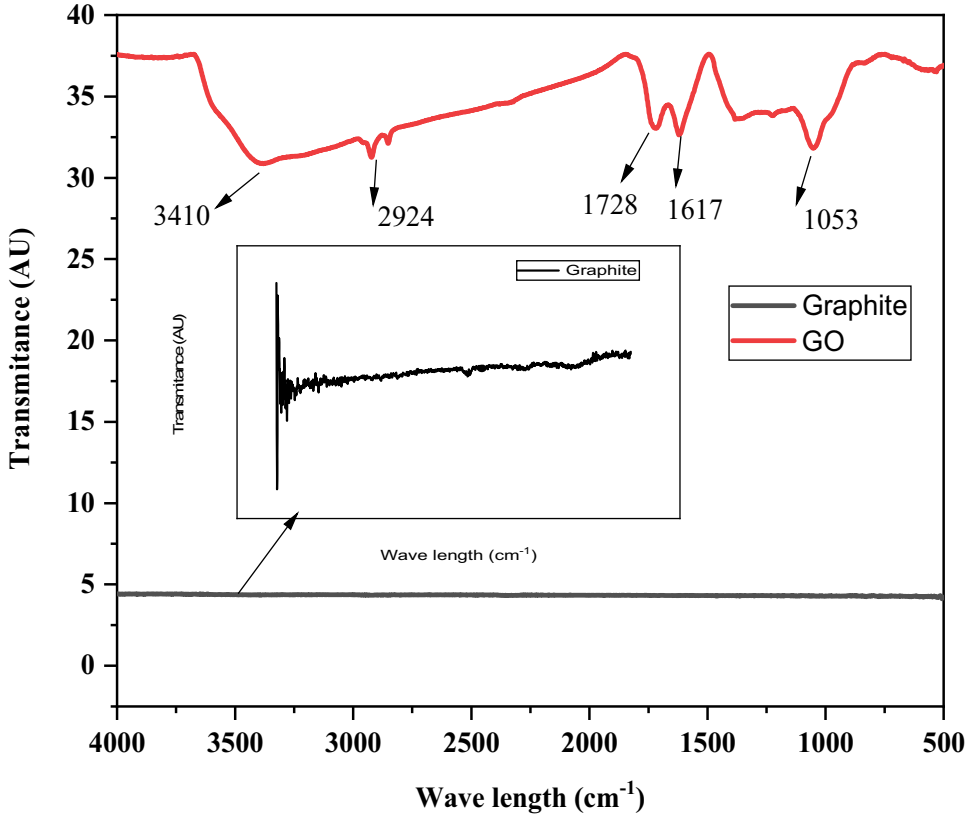


Figure 1. FT-IR spectrum of graphite and graphene oxide.

literatures [15,31]. The results of FT-IR spectrum of GO confirmed the successful chemical oxidation of the graphite during the oxidation process.

3.2. Optimisation of extraction conditions

In this experiment, parameters such as the amount of GO, amount of NaCl, sample pH, desorption solvent type and volume were investigated and optimised in order to achieve the best extraction efficiency for the triazine herbicides. The extraction performance of the proposed method was evaluated by spiking 0.5 ng mL^{-1} of the mixture triazines in 10 mL distilled water under different experimental conditions. All the experiments were performed in triplicate (experimental) and doublet reading (instrumental) and the mean value was taken as the optimum point.

3.2.1. Optimisation of the amount of GO adsorbent

The amount of GO on the peak area of the analyte compounds was studied in the range of 0.5–3 mg. The tests were performed following the procedure in Section 2.6. The results shown in Figure 2 indicate that the maximum peak area was achieved using 1 mg of GO for extraction of target analytes from 10 mL reagent water. This may be attributed to the chemical structure and high surface area of GO that increases the adsorption capacity [27,28]. Further increasing the amount of GO gave no improvement in the peak areas of

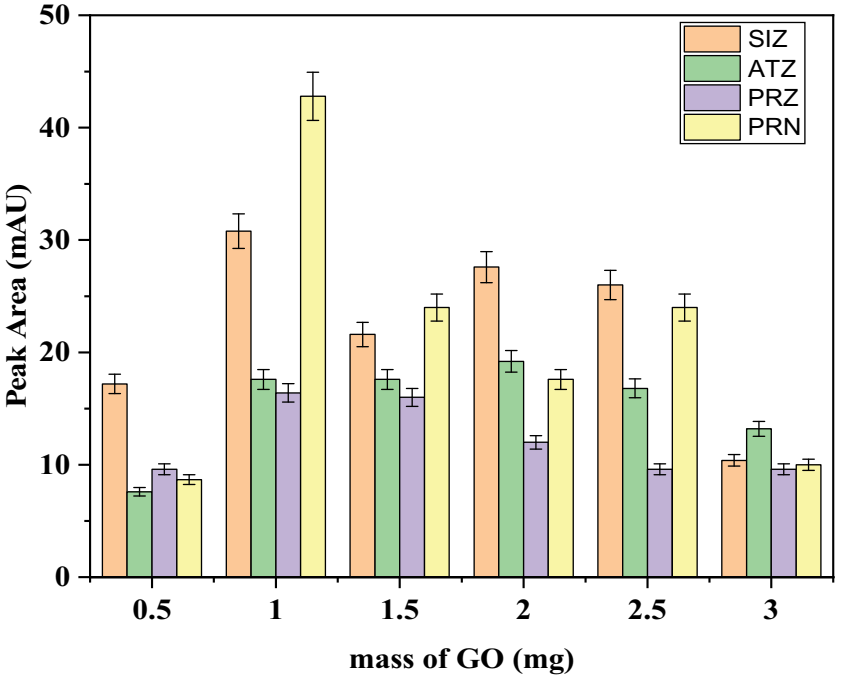


Figure 2. Effect of the amount of the graphene oxide (GO) nanoparticles on the peak areas of the triazines. Extraction conditions: sample volume, 10 mL; spiked concentration of the analytes, 0.5 mg L^{-1}; amount of NaCl, 29 mg; centrifuging time 10 min at 4,000 rpm; sample pH, 5; desorption solvent, 0.5 mL acetone.

the studied target analytes. It is important to note that more sorbent mass demands increased solvent requirements for desorption solvent and as a result may also reduce the resulting enrichment factor. Thus, 1 mg of GO was used in the subsequent studies as the optimum mass of GO.

3.2.2. Effect of pH

In solid phase extraction, pH is a parameter that plays a decisive role as it affects surface charge of sorbent, the extent of the ionisation and the solubility of the analytes in a sample solution. Thus, the surface chemistry of GO and the chemical structure of triazines are both dependent on the pH of the sample solution. The pH value of the solution would change the surface charge of GO by protonation/deprotonation transition of the hydroxyl and carboxyl functional groups; a primary factor which affects the adsorbing property of GO [32]. In addition, there are also π-π interactions between benzene rings of GO and the analytes, which is further favourable condition for the adsorption of triazines. In the present study, the effect of pH was investigated in the pH range of 3–8, since triazines are weak bases and they tend to protonate at excessively acidic pH media and hydrolyses at high basic pH conditions [33]. The pH value of the sample was adjusted by adding appropriate amounts of either 0.1 M HCl or 0.1 M NaOH solutions.

As can be seen from Figure 3, the peak areas exhibit increasing tendency by increasing the pH of the medium up to 5 and then decreased with further increase in pH. The pHpzc (point of zero charge) value of GO is about 3.9 [34]. Therefore, at the pH values lower than pHpzc, the surface charge of GO is positive because of the protonation reaction. In addition, the studied triazine pesticides have pK_a values in the range 1.65 to 4.1

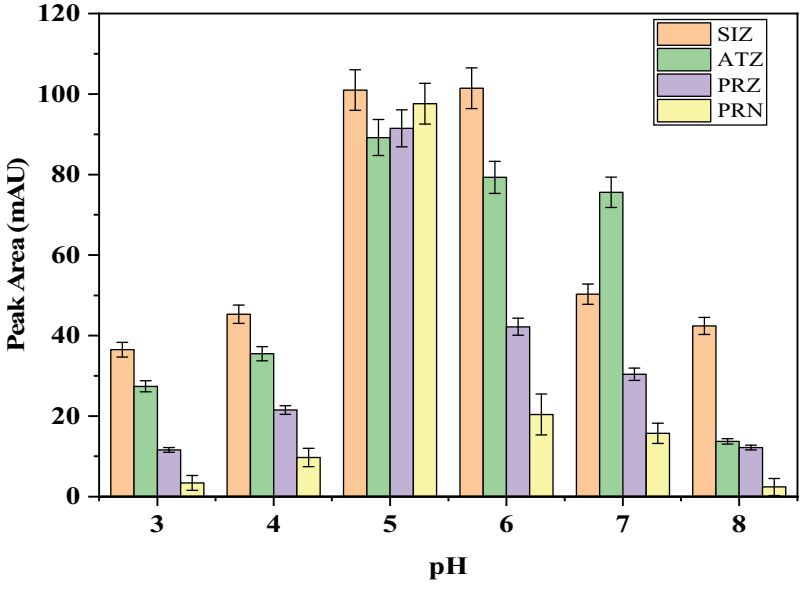


Figure 3. Effect of sample solution pH on the peak areas of the triazines. Extraction conditions: amount GO nanoparticles, 1 mg; sample volume, 10 mL; spiked concentration of the analytes, 0.5 mg L^{-1}; amount of NaCl, 29 mg; centrifuging time 10 min at 4,000 rpm; desorption solvent, 0.5 mL acetone.

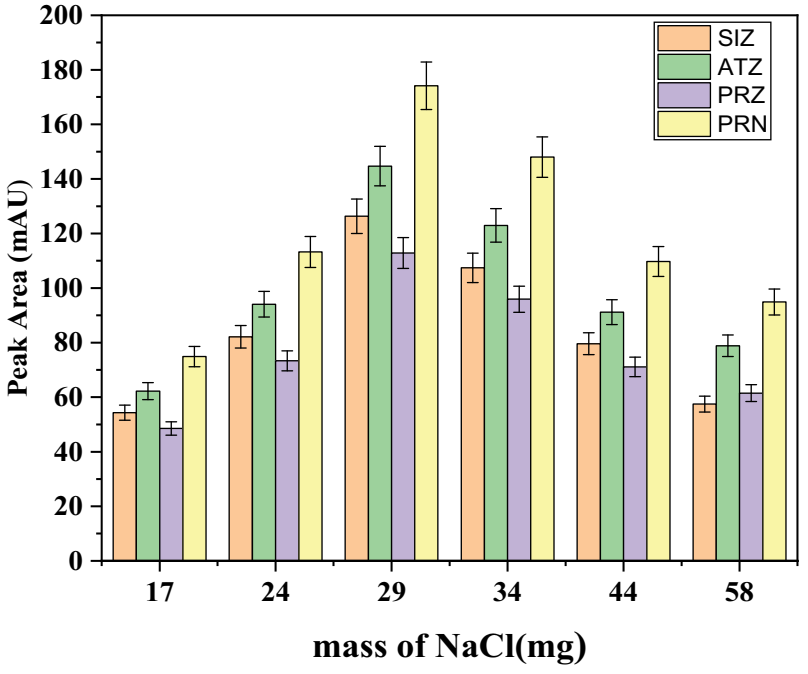


Figure 4. Effect of amount of NaCl on the peak areas of the triazines. Extraction conditions: amount GO nanoparticles, 1 mg; sample volume, 10 mL; sample solution pH, 5; spiked concentration of the analytes, 0.5 mg L^{-1}; centrifuging time 10 min at 4,000 rpm; desorption solvent, 0.5 mL acetone.

(Table 1), which are weak alkaline. Therefore, it is mostly protonated in strong acidic media [35,36]. As a result, they cannot bind well to the positively charged surface of the GO due to the electrostatic repulsion. As the surface charge of GO becomes negative, at pH values higher than the pHpzc, i.e. with increasing the pH value of solution, the electrostatic interactions between the GO and the triazine pesticides become stronger, which favours adsorption of triazines on the GO surface. The decrease in peak areas of triazines at pH values greater than 5 (Figure 3) may be due to the fact that more oxygen containing groups on the GO surface are deprotonated at the higher pH values [32]. This could be attributed to the adsorption of more water molecules on surface of GO and thus blocking the target analytes. Therefore, pH 5 was selected as optimum value for achieving the maximum adsorption of the studied analytes on the GO sorbent. Similar observations have also been noted by other workers [25].

3.2.3. The effect of NaCl

The effect of NaCl salt concentration on extraction efficiency was investigated using 5 ng mL^{-1} mixture of the triazines pesticides spiked in reagent water samples. Extraction efficiency increased when mass (in mg) of NaCl increased from 17 to 29 mg and decreased at higher concentrations of NaCl (Figure 4). Lower NaCl concentration resulted in insufficient GO aggregation and thus resulted in low efficiency of extraction of the analytes from aqueous phase. With higher NaCl concentration, insignificant elution of the analytes from GO resulted may be because of the increased viscosity of

the aqueous solution [28]. The achieved result can also be explained by the competition of higher concentration of Na^+ ions with the analyte compounds for extraction sites of GO sorbent [37]. Therefore, subsequent experiments were carried out with 29 mg NaCl.

3.2.4. Effect of desorption solvent type

Elution conditions are very important parameter to the efficiency of SPE, and the choice of solvent will greatly impact elution performance. In order to obtain a good recovery, elution of the analytes from the GO adsorbents was studied using common organic solvents which is available and inexpensive organic solvents such as methanol, acetonitrile, acetone and chloroform. The peak areas of the desorbed herbicides in these solvents were in the order of acetone > methanol > acetonitrile > chloroform (Figure 5). The eluting power of acetone was much stronger than the other three organic solvents, with chloroform exhibiting the least desorption performance. Therefore, acetone was the selected desorption solvent for this method. This could be attributed to the highest solubility of the target analyte compounds in acetone. Similar observations were also noted by other workers for elution of this class of pesticide compounds [12]. Moreover, acetone is preferred because of its low toxicity and is also inexpensive relative to the other solvents utilised during optimisation [38].

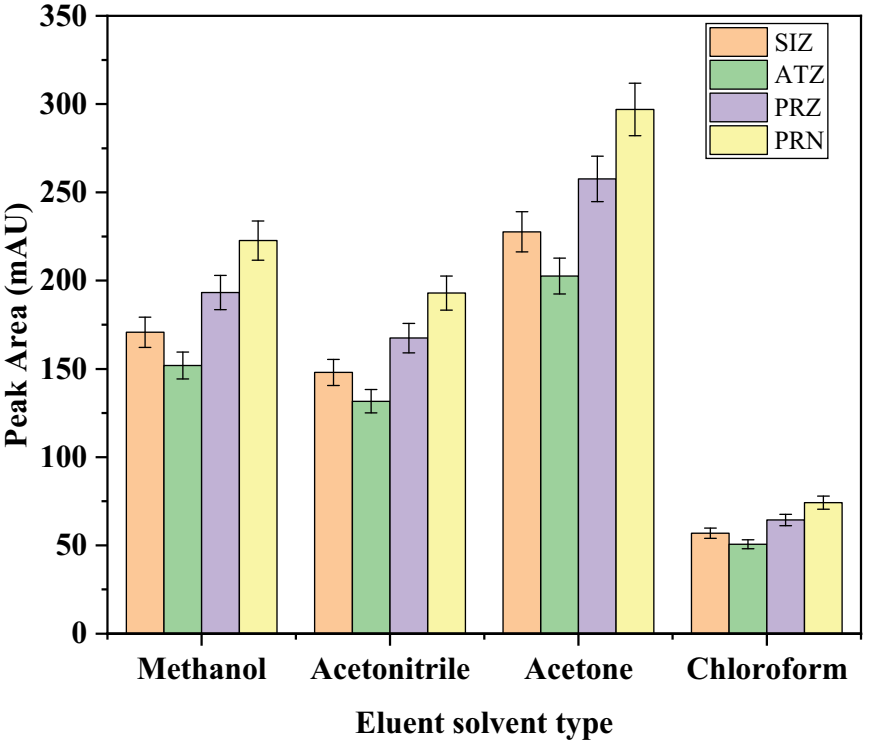


Figure 5. Effect of desorption solvent type on the peak areas of the triazines. Extraction conditions: amount GO nanoparticles, 1 mg; sample volume, 10 mL; amount of NaCl, 29 mg; sample solution pH, 5; spiked concentration of the analytes, 0.5 mg L^{-1}; centrifuging time 10 min at 4,000 rpm.

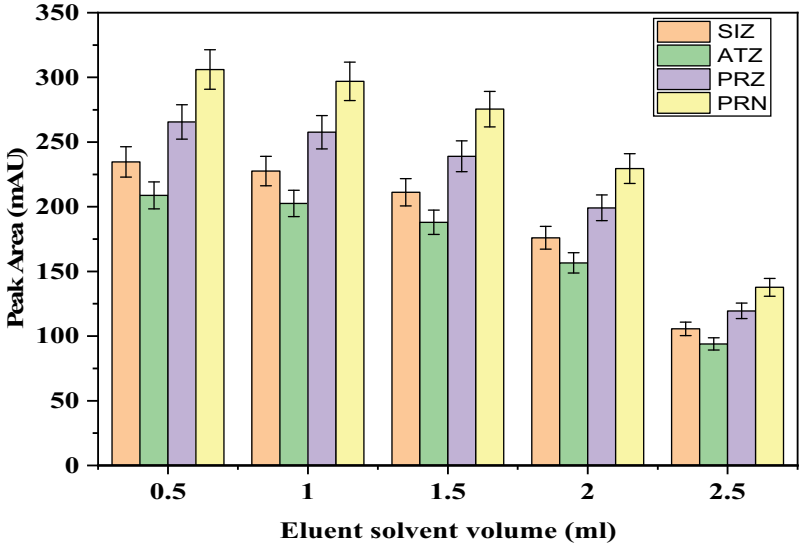


Figure 6. Effect of desorption solvent volume on the peak areas of the triazines. Extraction conditions: amount GO nanoparticles, 1 mg; sample volume, 10 mL; amount of NaCl, 29 mg; sample solution pH, 5; spiked concentration of the analytes, 0.5 mg L^{-1}; desorption solvent, acetone; centrifuging time 10 min at 4,000 rpm.

3.2.5. Desorption solvent volume

The volume of desorption solvent also has significant impact on the extraction efficiency. Thus, different volumes of the eluting solution ranging from 0.5 mL to 2.5 mL, with 0.5 mL interval were evaluated to have optimum volume. As indicated in Figure 6, the peak areas for the first two volume series appeared fairly similar, and then got lowered with increasing of the eluting solution volume. This could most probably be due to the dilution effect of the analytes concentration with increased eluent volume. Hence, 0.5 mL was taken as optimum eluent solvent volume. As described in Section 2.6, the eluate was collected and evaporated in oven dry at 35°C and then the residues were reconstituted with 300 µL mixture of acetonitrile and water (5:1 v/v), from which 20 µL was injected for HPLC analysis.

3.3. Analytical performance criteria of the method

To validate the applicability of the proposed SA-GO-DSPE method, after optimisation of the most influencing experimental conditions, all analytical performances of the method including limit of detection (LOD), limit of quantification (LOQ), linear range, correlation coefficient (r^2), accuracy and precision were determined. The developed SA-GO-DSPE method was first applied to the reagent aqueous solutions containing standard concentrations of the four triazine pesticides; in order to determine the linear range. The wide linear ranges observed in this analyses were in ng mL^{-1}; i.e. 5–1000 (for SIZ and PRZ), 2.5–1000 (for ATZ) and 8–1000 (for PRN), with correlation coefficient (r^2) 0.9991 or better. Then, calibration curves were obtained by considering the peak areas as the instrumental response versus the analyte concentrations. LOD and LOQ were calculated from the

Table 2. Analytical figures of merits for SA – GO-DSPE extraction technique combined with HPLC-DAD for the herbicide compounds under study in reagent water.

Analyte	Linear range (ng/mL)	Regression equation	LOD (ng/mL)	LOQ (ng/mL)	r^2	Repeatability (RSD %, n = 6)		Reproducibility (RSD%, n = 9)
SIZ	5 - 1000	y = 0.1864× + 3.0568	0.64	2.12	0.9993	5.4[a]	4.6[b]	6.27[b]
ATZ	2.5 - 1000	y = 0.143× + 3.3677	0.12	0.40	0.9992	3.29	2.54	4.38
PRZ	5 - 1000	y = 0.2039× + 4.3809	0.57	1.90	0.9991	2.77	4.56	7.94
PRN	8 - 1000	y = 0.2317× + 3.5017	0.80	2.68	0.9994	5.84	2.91	8.56

Note: [a]Concentration,10 ng mL^{-1}; [b] Concentration, 500 ng mL^{-1} ; Relative Standard Deviation (RSD).

standard deviation of the blank (σ) analysis and the slope of calibration curve (m) using the following equations: $\frac{3\sigma}{m}$ and $\frac{10\sigma}{m}$, respectively [1,17]. The analytical figures of merit for the optimised method are shown in Table 2. These data indicated that the proposed method has good sensitivity.

Precision of the proposed method was evaluated in terms of repeatability (intra-day precision) and reproducibility (inter-day precision). To study repeatability of the method, underground water sample spiked at two different concentrations (10 and 500 ng mL^{-1}) for the mixture of the four triazine pesticides were extracted in duplicate. The extract of each concentration was injected in triplicate on the same day under the optimised experimental conditions. Reproducibility of the method was also validated by using the same water sample used above to evaluate repeatability and spiked at the concentration of 500 ng mL^{-1}, for three consecutive days; following single extraction and triple injection. As shown in Table 2, the relative standard deviations (RSDs) of the method were between 2.54 and 5.84 for intra-day precision and 4.38 and 8.56 for inter-day precision; both results of the precision studies demonstrated good reliability of the proposed analytical method.

3.4. Applications of SA-GO-DSPE to real water analyses

Four different types of environmental waters including the tap, underground, spring, and river water samples were utilised for validation of the proposed method. Before spiking, the blank samples were analysed and the results showed that none of the four chosen target analytes were detected in any of the studied water samples. The observed results may designate either the water samples analysed were free from the residues of triazine herbicides or contained concentrations below the detection limits. The concentration may be below the detection limits due to their retention in solid soil mud as they have high log K_{ow} which leads to high adsorption capacity to solid soil mud [33]. The accuracy of the proposed SA-GO-DSPE method was evaluated from the relative recovery studies. To study the relative recovery (%RR), each water samples was spiked at three concentration levels (Table 3). Each concentration values were extracted in triplicate and then injected. Relative recovery was calculated as the ratio of the peak area of the spiked water samples to the peak area of the spiked ultrapure water sample [39]. Mean recoveries for the four triazines at three concentration levels were in the range of 69.06–95.53%, with %RSD from 1.32 to 6.99 in all kinds of environmental water samples studied. The results obtained for recovery were in acceptable range indicating that the proposed method has good

Table 3. The %RR and %RSD (n = 3) of the environmental water samples analysed for residue of triazine pesticides by SA-GO-DSPE method.

		Tap water		River water		Spring water		Ground water	
Triazine pesticides	Spiked concentration (ng/mL)	%RR	%RSD	%RR	%RSD	%RR	%RSD	%RR	%RSD
SIZ	0	nd	-	-	-	-	-	-	-
	10	88.08	2.81	69.40	5.03	85.76	5.01	76.92	6.38
	50	84.33	4.27	69.06	2.62	81.47	6.72	78.42	5.31
	500	85.67	2.84	79.23	4.34	89.59	4.25	79.23	4.58
ATZ	0	nd	-	-	-	-	-	-	-
	10	90.33	5.63	73.08	9.09	92.45	3.93	82.57	6.99
	50	88.54	4.81	76.00	6.84	86.31	2.98	83.59	1.62
	500	94.32	3.30	71.94	4.88	92.94	2.40	83.96	2.70
PRZ	0	nd	-	-	-	-	-	-	-
	10	92.43	5.89	73.56	7.52	84.82	4.68	79.39	7.82
	50	87.87	2.09	74.02	6.26	88.60	2.66	85.94	2.66
	500	95.53	2.82	75.53	4.17	89.44	4.11	86.83	2.18
PRN	0	nd	-	-	-	-	-	-	-
	10	91.97	5.57	72.30	5.01	86.49	1.73	78.31	5.03
	50	95.38	1.87	76.72	4.26	88.54	2.13	80.75	2.88
	500	92.85	3.34	75.55	4.26	91.23	1.32	80.08	1.96

nd: not detected

performances which were also similarly reported by other workers, both for accuracy and precisions [21,40].

The chromatograms of the target triazines in environmental water samples before and after spiking at the concentration of 40 ng mL^{-1} using the developed SA-GO-DSPE methods are shown in Figure 7. It is evident from this figure that there is no interfering peaks for the studied analytes at their respective retention time in the chromatograms. Therefore, the reported method is highly selective for the analyses of these and similar other triazine herbicides in real water samples.

3.5. Comparison of the SA-GO-DSPE method with other SPE methods in the literature

The established method, SA-GO-DSPE using GO as sorbent followed by HPLC-DAD analysis, was compared with other published methods for the determination of triazines in environmental water samples. The results are summarised in Table 4. The reported detection techniques compared includes, stir bar sorptive extraction (SBSE) coupled with gas chromatography mass spectrometry (GC-MS) [41], solid phase membrane tip extraction (SPMTE) which involving the use of tiny cone shaped membrane tip protected multiwall carbon nanotubes (MWCNTs) combined with micro liquid chromatography (Micro LC-UV) [42], dispersive micro solid phase extraction with ultrahigh performance liquid chromatography high-resolution mass spectrometric detection (DMSPE -UHPLC-HRMS) [43], SPE-HPLC-DAD using hydrophilic lipophilic balanced (HLB) sorbent [33,44], micro solid phase extraction using titanium dioxide (TiO_2) nanotube array as sorbent (μ-SPE-HPLC-DAD) [45] and sol-gel poly (ethylene glycol)(PEG) coated stir fabric phase sportive extraction (SFPSE) device in combination with UHPLC-DAD (SFPSE-UHPLC-DAD) [46]. As can be seen, in comparison with other reported methods, the proposed method had the wider linear range, and comparable or the lowest detection limit and good

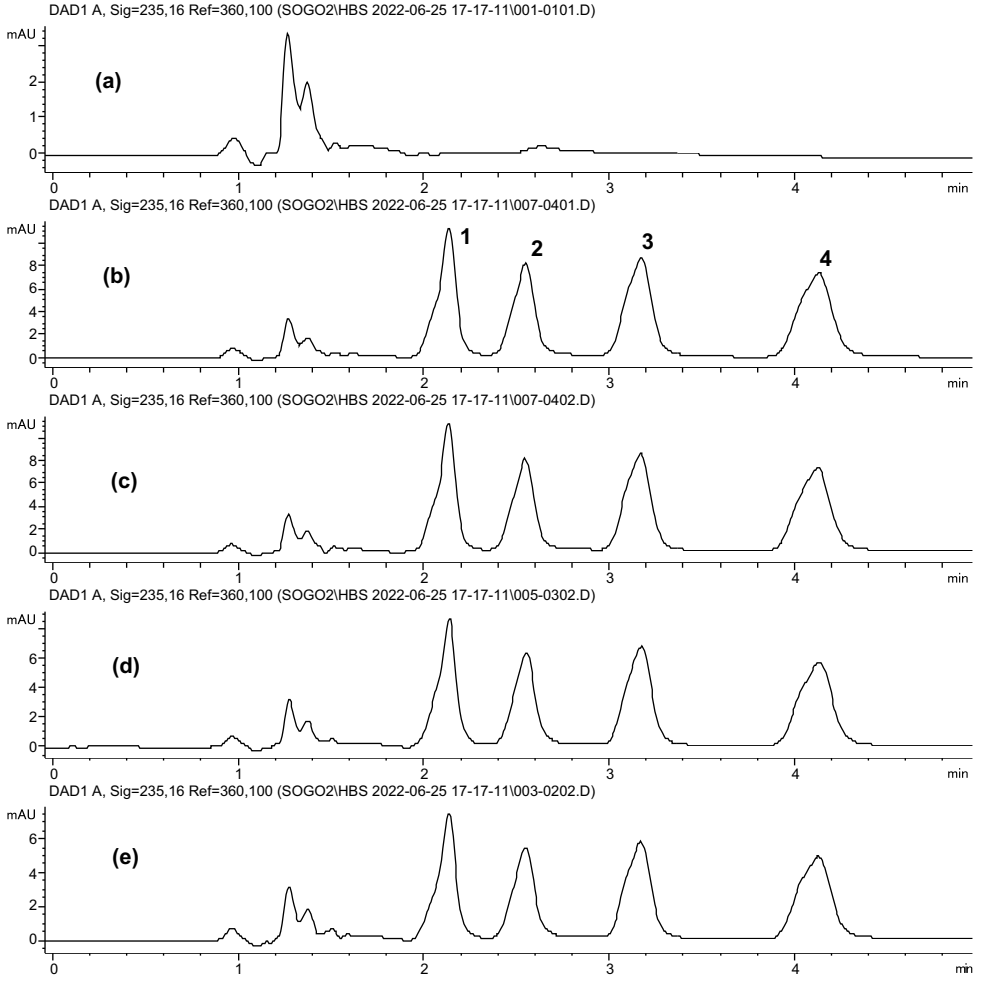


Figure 7. Typical chromatograms for blank (a) and spikedtap water (b), spiked spring water (c), spiked underground water (d) and spiked river water (e) samples. Extraction conditions: amount GO nanoparticles, 1 mg; sample volume, 10 mL; amount of NaCl, 29 mg; sample solution pH, 5; spiked concentration of the analytes, 40 ng mL^{-1} ; desorption solvent volume, 0.5 mL; centrifuging time 10 min at 4,000 rpm. Peaks: 1. simazine; 2. atrazine; 3. propazine; 4. prometryn.

precision. Although SBSE-GC-MS and DMSPE-UHPLC-HRMS have better LOD values, the detection methods applied were high tech complex instruments which need skilled manpower and expensive to use in routine laboratory analysis for developing country like Ethiopia. Additionally, as sorbent used in some reported methods are commercially prepared, expensive and not available routine laboratories. Preparation of other sorbents compared with GO are also time-consuming procedure, require many organic and in organic compounds which are not cost effective and ecofriendly method. Therefore, the developed method is a valuable alternative to the routine analytical methods for extraction of triazine pesticides.

Table 4. Comparison of the proposed method with other solid phase extraction form methods applied for the extraction and determination of triazine pesticides in water samples.

Method	Sorbent	Detection	LR (μg L^{-1})	LOD (μg L^{-1})	RSD	Ref
SBSE	PDMS	GC-MS	0.01-10	0.0012–0.0034	1.5–5.1	[41]
SPMTE	MWCNT	Micro-LC-UV	1-100	0.2–0.5	6-8	[42]
DMSPE	PCX	UHPLC-HRMS	0.01-20	0.0002–0.03	<12.7	[43]
SPE	HLB	HPLC-DAD	-	0.668–1.6	1-18	[33]
SPE	HLB	LC-DAD	200-1000	0.026–0.084	0.24–0.36	[44]
μ-SPE	TiO_2	HPLC-UV	1.0-200	0.19–0.50	2.3–4.2	[45]
SFPSE	PEG	UHPLC-DAD	-	0.08–0.24	1.4–11.7	[46]
DSPE	GO	HPLC-DAD	2.5-1000	0.12–0.80	1.32–9.09	This work

Linear range (LR); Solid phase membrane tip extraction (SPMTE); Hydrophilic lipophilic balanced (HLB); Stir bar sorptive extraction (SBSE); Poly (ethylene glycol) (PEG); Stir fabric phase sorptive extraction (SFPSE).

4. Conclusion

In the present study, a GO nanomaterial was synthesised and used successfully as SPE sorbent. The developed analytical method based on the SA-GO-DSPE combined with HPLC-DAD was applied for selective sample extraction and simultaneous quantitative determination of the most commonly used four triazine pesticides at trace level in water samples. When the method applied to the extraction of triazines from real water samples such as tap, spring, underground and river water; no matrix interferences were coextracted and observed in the analysis in liquid chromatographic separation. The sorbent used as nanoparticle was observed to possess high adsorption capacity and rapid adsorption rates. In the experimental procedure, it was realised that only low amount of sorbent can be used and also short equilibrium time was required to extract the analytes from samples. Hence, the method has the advantages of short analysis time, simplicity, easy operation and low organic solvent consumption; which implies that the methods in agreement with the principles of Green Chemistry, suggesting the established method combined with HPLC-DAD could be successfully applied as a rapid alternative for preconcentration, extraction, and determination of triazine pesticides in environmental water samples and other trace organic pollutants in routine laboratories analysis.

Acknowledgments

The authors would like to thank the Department of Chemistry, Addis Ababa University, for continuous assistance in providing laboratory facilities and the required consumables. Habtamu Bekele is grateful to the Oromia Regional State Education Bureau for the sponsorship granted for his PhD study.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The authors declare that no funds, grants, or other support were received during this research work.

Data availability statement

All the data are included in the manuscript. There are no additional data with the authors.

References

[1] L. Zhang, R. Yu, Z. Wang, N. Li, H. Zhang and A. Yu, J. Chromatogr. B. **954**, 132 (2014). doi:10.1016/j.jchromb.2014.02.011.
[2] N. Megersa, Anal. Methods **7**, 9940 (2015). doi:10.1039/c5ay01927c.
[3] H.M. Mnyandu and P.N. Mahlambi, Food Chem. **360**, 29818 (2021). doi:10.1016/j.foodchem.2021.129818.
[4] J. Ilavský, D. Barloková and M. Marton, IOP Conf. Ser. Earth Environ. Sci. **609**, 012088 (2020). doi:10.1088/1755-1315/609/1/012088.
[5] A.L. De Toffoli, B.H. Fumes and F.M. Lanças, J. Environ. Sci. Heal. Part B. 1 (2018). doi:10.1080/03601234.2018.1438831.
[6] N. Megersa and J.Å. J¨onsson, Analyst. **23**, 225 (1998). doi:10.1039/A705361D.
[7] R. de Prá Urio and J.C. Masini, Anal. Lett. **50**, 1065 (2017). doi:10.1080/00032719.2016.1212203.
[8] P. Chen, W. Haung and S. Huang, J. Chromatogr. B. **955**, 116 (2014). doi:10.1016/j.jchromb.2014.02.032.
[9] H. Sabik and R. Jeannot, J. Chromatogr. A. **818**, 197 (1998). doi:10.1016/S0021-9673(98)00555-X.
[10] Z. Shi, S. Zhang, Q. Huai, D. Xu and H. Zhang, Talanta. **162**, 300 (2017). doi:10.1016/j.talanta.2016.10.042.
[11] X. Zhang, X. Ma, X. Li, C. Li, R. Wang and M. Chen, Water Air Soil Pollut. **229**, 270 (2018). doi:10.1007/s11270-018-3930-y.
[12] H. Zhang, Y. Yuan, Y. Sun and C. Niu, Analyst. **143**, 175 (2017). doi:10.1039/c7an01290j.
[13] H. Sereshti, M. Khosraviani, S. Samadi and M.S. Amini-Fazl, RSC Adv. **4**, 47114 (2014). doi:10.1039/c4ra06412g.
[14] Z. Shi, J. Hu, Q. Li, S. Zhang, Y. Liang and H. Zhang, J. Chromatogr. A **1355**, 219 (2014). doi:10.1016/j.chroma.2014.05.085.
[15] M. Foschi, P. Capasso, M.A. Maggi, F. Ruggieri and G. Fioravanti, ACS. Omega. **6**, 16943 (2021). doi:10.1021/acsomega.1c01877.
[16] G. Zhao, S. Song, C. Wang, Q. Wu and Z. Wang, Anal. Chim. Acta **708**, 155 (2011). doi:10.1016/j.aca.2011.10.006.
[17] H.R. Nodeh, W.A. Wan Ibrahim, M.M. Sanagi and H.Y. Aboul-Enein, RSC Adv. **6**, 24853 (2016). doi:10.1039/c5ra26742k.
[18] Q. Xu, K. Qiao, C. Yan, Z. Liu, R. Lu and W. Zhou, Anal. Methods. **12**, 3115 (2020). doi:10.1039/d0ay00562b.
[19] M. Asadi, H. Sereshti and H. Rashidi Nodeh, Res. Chem. Intermed. **46**, 2789 (2020). doi:10.1007/s11164-020-04121-y.
[20] M. Mohammadnia, R. Heydari and M.R. Sohrabi, J. Environ. Sci. Heal. Part B. **55**, 293 (2020). doi:10.1080/03601234.2019.1692613.
[21] W. Song, Y. Zhang, G. Li, H. Chen, H. Wang, Q. Zhao, D. He, C. Zhao and L. Ding, Food Chem. **143**, 192 (2014). doi:10.1016/j.foodchem.2013.07.101.
[22] T. Gao, J. Wang, Q. Wu, C. Wang, Z. Wang and Z. Wang, Food Anal. Methods. **13**, 90 (2020). doi:10.1007/s12161-019-01685-3.
[23] S. Mahpishanian and H. Sereshti, J. Chromatogr. A. **1443**, 43 (2016). doi:10.1016/j.chroma.2016.03.046.
[24] P. Sun, Y.L. Gao, C. Xu and Y.F. Lian, RSC Adv. **8**, 10277 (2018). doi:10.1039/C7RA13316B.
[25] P.K. Boruah, B. Sharma, N. Hussain and M.R. Das, Chemosphere. **168**, 1058 (2017). doi:10.1016/j.chemosphere.2016.10.103.

[26] M. Anastassiades, K. Maštovská and S.J. Lehotay, J. Chromatogr. A. **1015**, 163 (2003). doi:10.1016/S0021-9673(03)01208-1.
[27] D. Deng, X. Jiang, L. Yang, X. Hou and C. Zheng, Anal. Chem. **86**, 758 (2013). doi:10.1021/ac403345s.
[28] G. Li, X. Zhang, L. Zhang, S. Xu and C. Li, Chromatographia. **78**, 979 (2015). doi:10.1007/s10337-015-2913-z.
[29] N.I. Zaaba, K.L. Foo, U. Hashim, S.J. Tan, W.W. Liu and C.H. Voon, Procedia Eng. **184**, 469 (2017). doi:10.1016/j.proeng.2017.04.118.
[30] P.K. Boruah, G. Darabdhara and M.R. Das, Chemosphere **268**, 129328 (2021). doi:10.1016/j.chemosphere.2020.129328.
[31] R.D.S. Antônio, A. Carolina, S. Guerra, M.B. De Andrade and L. Nishi, Environ. Sci. Pollut. Res. **28**, 5731 (2021). doi:10.1007/s11356-020-10693-4.
[32] S. Zeng, N. Gan, R. Weideman-Mera, Y. Cao, T. Li and W. Sang, Chem. Eng. J. **218**, 108 (2013). doi:10.1016/j.cej.2012.12.030.
[33] P.N. Kunene and P.N. Mahlambi, J. Environ. Manage. **249**, 109415 (2019). doi:10.1016/j.jenvman.2019.109415.
[34] S. Mahpishanian and H. Sereshti, Talanta. **130**, 71 (2014). doi:10.1016/j.talanta.2014.06.004.
[35] Y.L. Du and C.P. Pan, ACS Sustain. Chem. Eng. **3**, 396 (2015). doi:10.1021/sc500738k.
[36] E. Teju, B. Tadesse and N. Megersa, Sep. Sci. Technol. **6395**, 1 (2017). doi:10.1080/01496395.2016.1276596.
[37] S. Zhu, Y. Liu, F. Qian, C. Zhou, S. Zhang and J. Chen, Chemosphere. **179**, 20 (2017). doi:10.1016/j.chemosphere.2017.03.071.
[38] M. Shamsipur, N. Fattahi, M. Pirsaheb and K. Sharafi, J. Sep. Sci. **35**, 2718 (2012). doi:10.1002/jssc.201200424.
[39] X. Ma, J. Wang, Q. Wu, C. Wang and Z. Wang, Food Chem. **157**, 119 (2014). doi:10.1016/j.foodchem.2014.02.007.
[40] T. Tolcha, Y. Merdasa and N. Megersa, J. Sep. Sci. **36**, 1119 (2013). doi:10.1002/jssc.201200849.
[41] A. Sanchez-Ortega, N. Unceta, A. Gómez-Caballero, M.C. Sampedro, U. Akesolo, M.A. Goicolea and R.J. Barrio, Anal. Chim. Acta. **641**, 110 (2009). doi:10.1016/j.aca.2009.03.044.
[42] H.H. See, M. Marsin Sanagi, W.A.W. Ibrahim and A.A. Naim, J. Chromatogr. A. **1217**, 1767 (2010). doi:10.1016/j.chroma.2010.01.053.
[43] D. Chen, Y. Zhang, H. Miao, Y. Zhao and Y. Wu, J. Agric. Food Chem. **63**, 9855 (2015). doi:10.1021/acs.jafc.5b03973.
[44] S.C. Ntombela and P.N. Mahlambi, J. Environ. Sci. Heal. Part B. **54**, 569 (2019). doi:10.1080/03601234.2019.1621113.
[45] Q. Zhou and Z. Fang, Anal. Methods. **7**, 3277 (2015). doi:10.1039/c4ay03012e.
[46] M. Roldán-Pijuán, R. Lucena, S. Cárdenas, M. Valcárcel, A. Kabir and K.G. Furton, J. Chromatogr. A. **1376**, 35 (2015). doi:10.1016/j.chroma.2014.12.027.

Paper - II

(Published)

Fast surface floating organic droplets based dispersive liquid-liquid microextraction for trace enrichment of multiclass pesticide residues from different fruit juice samples followed by high performance liquid chromatography–diode array detection analysis

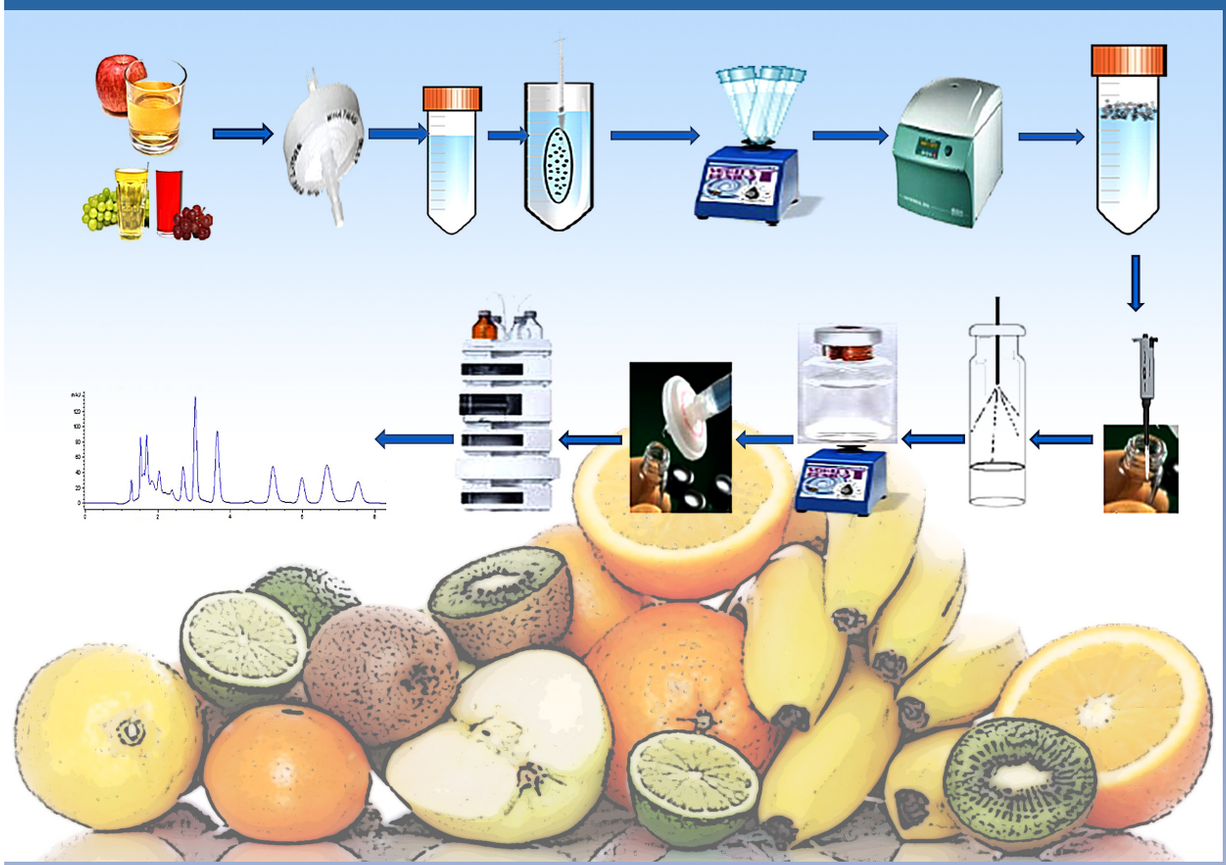
Habtamu Bekele and Negussie Megersa

Separation Science Plus, 6(8), 2023, 1-12. **https://doi.org/10.1002/sscp.202300042**

ISSN 2573-1815 · SSC 6 (8) 2023 · Vol. 6 · No. 8 · August 2023

SSC plus

SEPARATION SCIENCE PLUS



Methods
Chromatography · Electroseparation

Applications
Biomedicine · Foods · Environment

8|23

WILEY VCH

www.sscp-journal.com

Received: 28 March 2023 | Revised: 23 May 2023 | Accepted: 26 May 2023

DOI: 10.1002/sscp.202300042

RESEARCH ARTICLE

Fast surface floating organic droplets based dispersive liquid-liquid microextraction for trace enrichment of multiclass pesticide residues from different fruit juice samples followed by high performance liquid chromatography–diode array detection analysis

Habtamu Bekele | **Negussie Megersa**

Department of Chemistry, College of Natural and Computational Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Correspondence
Habtamu Bekele and Negussie Megersa, Department of Chemistry, College of Natural and Computational Sciences, Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia.
Email: habtamu.bekele@aau.edu.et; negussie.megersa@gmail.com

This study was designed to enable the development of a simple, fast, and environmentally friendly analytical technique utilizing dispersive liquid-liquid microextraction based on surface floating organic droplets for selective and quantitative enrichment of trace level pesticide contaminants from different fruit juice samples for subsequent detection by high performance liquid chromatography, combined with a diode array detector. The selective extraction was necessitated in order to isolate the seven multiclass pesticide residues frequently occurring in fruit juice samples. The effects of experimental parameters such as pH of sample solution, type and volume of extraction and dispersive solvents, ionic strength and extraction time were optimized. The optimized method was validated using spiked blank sample and satisfactory results for accuracy, with recoveries ranging from 87.23% to 99.45%, with %relative standard deviation between 1.37 and 8.39, precision in terms of %relative standard deviation $\leq$ 10.78 and linearity at concentration levels from 3 to 1500 ng/ml, which corresponded with correlation coefficients $\geq$ 0.998. The limits of detection and the limits of quantification were ranged from 1.3×10^{-2} to 5.3×10^{-2} and 4.2×10^{-2} to 1.8×10^{-1} μg/L, respectively. At the end, the method was successfully applied to analyze real fruit juice samples and target analytes were not detected in real samples.

KEYWORDS
Azoxystrobin, fruit, high performance liquid chromatography, pesticide residues, trace enrichment

1 | INTRODUCTION

Pesticides are chemical substances used to eradicate weeds, plant diseases, insects, fungi, and other pests that could harm fruits and subsequently cause lower agricultural yields. They are manufactured from multiple molecule substances obtained from various chemical groups that share some characteristics [1]. Out of the total

Article Related Abbreviations: DAD, diode array detection; DLLME–SFOD, dispersive liquid-liquid microextraction-based surface floating organic droplet; LPME, liquid-phase microextraction; MeOH, methanol.

amount of pesticides sprayed on the plants, only less than 0.1% of the applied pesticides actually reach the targeted pests; the remainder may end up on other environmental compartments and consumables like fruits and vegetables [2–5]. This has led to the need for discovery of these compounds in food, soil, and water, possibly compromising both human life and health of all other living species [6]. Due to their toxicity and bioaccumulation, it is essential and necessary condition to perform routine monitoring of the residues of pesticides in food and similar other matrices utilized in the livelihood of humans [4, 7].

For the purpose of quantifying and separating various pesticide residues, in complex matrices, numerous chromatographic techniques have commonly been employed. Most pesticides are volatile and thermally stable, making them suitable for GC [8–13]. On the other hand, HPLC coupled with a diode array detector (DAD) is also frequently used to determine polar and thermally unstable pesticide compounds [7, 14–21]. Both LC−MS and LC−MS/MS procedures, providing high sensitivity and better levels of selectivity, have increased the interest of researchers these days [4, 22]. Most common laboratories lack expensive analytical instruments and have difficulties accessing modern equipment such as LC−MS and LC−MS/MS. Therefore, it seems appealing and encouraging to develop sensitive and effective analytical procedures that use HPLC−DAD for routine assessments of multiclass pesticide residues, especially in the labs of the less developed world.

Nowadays, sample preparation is mandatory before the analysis, despite the invention of a variety of very sensitive instrumental methods of analysis. This is because some real sample matrices are complex, incompatibility with the analytical instrument, making the analysis of multiclass pesticide residues in foods difficult, as the concentrations at which they typically occur are very low. This is known to be an obvious challenge to accurately separating the analytes of interest and detecting them, to the required degree of precision, mainly because of the wide range of differences in chemical and physical properties. The difficulty is more serious when the pesticide residues are mixtures from multiple classes, which complicates the chemistry, and thus a search for efficient extraction combined with clean-up analytical procedures is critically needed [9, 17]. Conventional sample preparation techniques, such as liquid-liquid extraction [23], SPE [24], and cloud point extraction [25] are methods used for selectively isolating multiclass pesticides for accurate determination in food samples. These methods should enable boosting sensitivity for determining trace level determination of the target analytes from sample matrices of varying origin. However, some of these techniques owe quite a number of drawbacks including the need for huge volumes of costly and hazardous organic solvents, labor-intensive processes, prolonged extraction, and so forth [19, 26]. Furthermore, miniaturization of the extraction methods was subsequently introduced for trace level enrichment such as SPME [27], magnetic SPE [10], single drop microextraction [28], stir bar sorptive extraction [6], continuous sample drop flow-microextraction [29] and hollow fiber liquid-phase microextraction (LPME) [30] and frequently utilized for trace analyses of food samples. Advantages and drawbacks of these techniques were explained in details by other workers [31]. To overcome some of the drawbacks encountered in both traditional and miniaturized sample preparation techniques, one of these methods developed is the dispersive liquid-liquid microextraction (DLLME), which gradually received attention in the recent years.

DLLME is a miniaturized LPME technique that uses a ternary component system made up of the aqueous phase, extraction solvent, and disperser solvent [32]. Due to the turbid suspension that is produced by this method and the substantial surface area that is created between the aqueous phase and extraction solvent, analytes transfer from the aqueous phase to the extraction solvent was found easy. Different modes of DLLME for preconcentration of multiple pesticide residues in a variety of food matrices such as vegetables and fruit juices have become widely used in recent years as a result of the replacement of traditional sample preparation techniques with DLLME [18, 19, 33]. Although conventional DLLME, which commonly uses chlorinated solvents, has many advantages including ease of use, speed, affordability, high recoveries, and a high enrichment factor [9], the widespread use of halogenated solvents as extraction solvents has been constrained by their toxicity, which is also linked to human cancers. As a result, usage of chlorinated solvents has been outlawed globally and gradually been replaced with more environmentally friendly solvents that are lighter in density than water to address these shortcomings [34]. Moreover, in DLLME based surface floating organic droplet (DLLME−SFOD) technique collection of extraction solvent seems to be interesting and easy task for the extraction of multiresidue compounds [8]. After its development, numerous research works have been reported for analysis of pesticides in foods including fruit juices [17, 29, 35–40]. However, to date there is no literature report on the use of DLLME−SFOD procedure, in combination with HPLC−DAD, for simultaneous analysis of the commonly used pesticides such as carbamate (carbrayl), organophosphorus (dimetathote and metadiothate), triazines (cyanazine and atrazine), phenyl urea (linuron) and strobilurin (azoxystrobin) pesticides in fruit juice samples.

Therefore, despite the considerable development of sample preparation techniques in recent years, multiresidue methods using microextraction techniques are difficult to develop because compounds with different physico-

chemical properties must be extracted simultaneously. It is obvious that this method is more environmentally friendly; reducing the cost and time compared to the methods that extract a single class of pesticide pollutants. In this study, a DLLME–SFOD combined with HPLC–DAD has thus been proposed for trace level enrichment of seven multiclass pesticide residues stated above in selected fruit juice samples. Deferent experimental parameters affecting the extraction efficiency of the target analytes were studied and the optimal conditions have been established. The analytical performances and possible applications in fruit juices were also investigated in order to confirm the reliability of the method for the target analytes.

2 | EXPERIMENTAL SECTION

2.1 | Chemicals and reagents

Two of the analytical standards considered in this study, methidathion and dimethoate insecticides and hydrochloric acid (HCl) were purchased from Sigma Aldrich (St. Louis, MO, USA). Two chloro-*s*-triazines including atrazine and cyanazine were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Azoxystrobin, carbaryl and linuron were obtained from Sigma Aldrich (Steinheim, Germany). All the standards were of the highest purity, that is, > 98 %. Other chemicals used in the study were of analytical grade reagents while the solvents utilized including ACN and acetone, acquired from Sigma Aldrich (Steinheim, Germany), methanol (MeOH) received from Carlo Erba (Rodano, Italy) and isopropanol (IPA) the product of Sigma Aldrich (Seelze, Germany) were of HPLC grade reagents. Common chemicals such as NaCl was obtained from Sigma Aldrich (Steinheim, Germany) and sodium hydroxide (NaOH) was the product of Merck Chemicals (Darmstadt, Germany). Ultrapure water was obtained by purifying with double distiller, A 8000 Aquatron water Still (Bibby Scientific, Staffordshire, UK) and deionizer (EASY Pure LF, Dubuque).

2.2 | Instruments and equipment

Chromatographic analyses were carried out using Agilent 1200 series HPLC system (Agilent Technologies, Waldbronn, Germany) outfitted with a quaternary pump, vacuum degasser, standard and preparative autosampler, thermostated column compartment, autosampler thermostat, and a diode array multiple wavelength detector. LC Chemstation software (B.02, 01-SR1) was used for sample processing and data acquisition. Chromatographic separation was performed using a ZORBAX ODS-C_{18} (150 × 3 mm, i.d., 3.5 μm particle size) analytical column from Agilent Technologies. The sample solution pH was measured using an Adwa pH meter, model 1020, made by Adwa Hungary Kft, in Szeged, Hungary. For sample preparation, a centrifuge, Model 800, Jiangsu Zhenji Instruments Ltd. (Jiangsu, China), a 15 ml centrifuge tube, Corning integrated (Corning, NY, Mexico), and an ultrasonic heater, Dacon, were utilized.

2.3 | Chromatographic conditions

Chromatographic separations were achieved using isocratic delivery mode a binary mobile phase, consisting of solvent A (ultrapure water) and solvent B (MeOH) in a 2:3 volume ratio, respectively. Prior to the sample injection, the HPLC column was equilibrated with the mobile phase for 10 min. Analysis was performed with the flow rate of 1 ml/min, column temperature at 35°C, injection volume of 15 μl and UV detection was performed at 224 nm for all the target analytes. Peak area was used as instrumental response and comparison of the responses. Under these chromatographic conditions, baseline separation was maintained for all the target analytes.

2.4 | Standard solution preparation

The stock standard solution of each target analyte, with a concentration of 100 μg/ml, was prepared by weighing appropriate amount and dissolving it in MeOH. Intermediate standard solutions of 10 μg/ml were obtained by diluting the stock solution with ultrapure water. Other working solutions of lower concentrations were also prepared from the intermediate solution in the ultrapure water. All standard solutions were stored in the refrigerator below 4°C, when not in use. The chemical structure, common name, abbreviation and the octanol water partition coefficient (log *P*) at pH 7 and 20°C, and other relevant physicochemical properties of the target pesticides are shown in Figure 1.

2.5 | Juice samples

Various fruit juice samples including the orange, apple, grape, pineapple, and guava were considered in the presented study and were obtained both from local supermarkets, in the city of Addis Ababa and imported from abroad. Samples of fruit juice that are containing no pesticide residues (blank samples) were utilized during the recovery studies. All fruit juice samples were kept in their original packaging and stored in dark drawers at room temperature, when not in use.

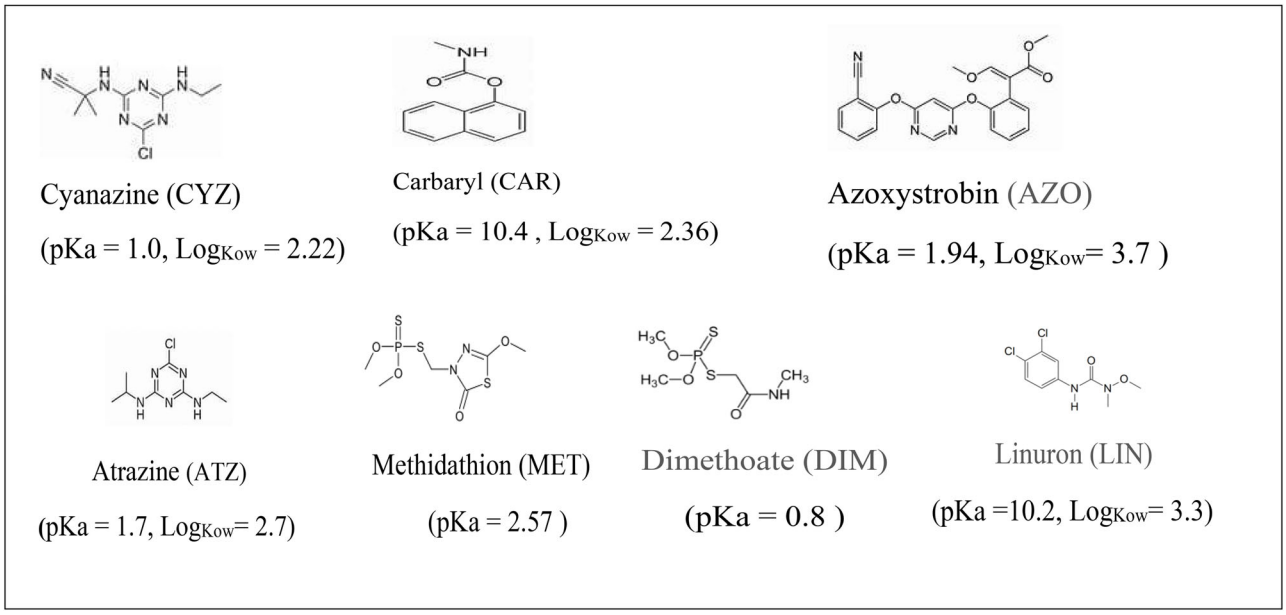


FIGURE 1 Chemical structure, common name, pK_a, and log K_{ow} (partition coefficient) of the target pesticides considered in this study.

2.6 | DLLME–SFOD extraction procedure

Fruit juice samples were filtered through a 0.45 μm micropore membrane syringe filter and 5 ml portions of each kind of fruit juice sample, adjusted to pH 5, was measured and transferred into a 15 ml falcon tube with conical bottom. Afterwards, it was fortified with appropriate amount of mixture of each target analytes and left to stand for about 10 min for equilibration. Then, before using the sample for DLLME, aqueous solution of 15% (w/v) NaCl was added and mechanically agitated for 1 min to dissolve in the juice sample. Subsequently, the organic phase consisting of a mixture of 1 ml ACN and 100 μl toluene was injected into the sample solution with a syringe attached to a needle with a flat point. Using a micropipette, the surface-floating organic phase of the extract was carefully collected and transferred into a 1.5 ml vial, where the extraction solvent was evaporated to dryness at room temperature. The residue was then reconstituted with 300 μl of MeOH, vortex agitated for 1 min and then filtered through a 0.45 μl micropore membrane filter before injecting to the HPLC system for analysis.

3 | RESULTS AND DISCUSSION

3.1 | Optimization of the DLLME–SFOD

This research work was designed with the interest to develop an efficient analytical methodology that is miniaturized, simple, fast, and cost-effective for the analysis of multiresidue pesticides. Attainment of the desired efficiency was achieved by making use of a single sample preparation process to be able to analyze seven multiresidue pesticides simultaneously. The effects of crucial experimental parameters that may have impacts on the DLLME extraction efficiency were investigated and optimized using the univariate approach in order to reach a reliable extraction efficiency. All the experiments were performed at least in triplicate (experimental) and doublet reading (instrumental), and the mean instrumental reading result, in each parameter study, was taken as the optimum value. Peak area was taken as an instrument response, for establishing the optimum experimental condition for each parameter under study.

3.1.1 | Effect of the extraction solvents

The selection of the appropriate extraction solvent is one of the major steps for the DLLME–SFOD process. The solvent of choice, in this study, is one that has a lower density than and is immiscible with water but miscible with dispersive solvents. To this end, five organic solvents including octanol (0.827 g/ml), heptane (0.680 g/ml), toluene (0.865 g/ml), dihexyl ether (0.785 g/ml), and undecane (0.740 g/ml), were tested for use as extraction solvent. As has also been indicated in Figure 2, toluene has provided the highest peak areas for most of the analyte compounds considered in this study, and thus was chosen as the preferred extraction solvent. Toluene was similarly used, as extraction solvent, for selective extraction of other

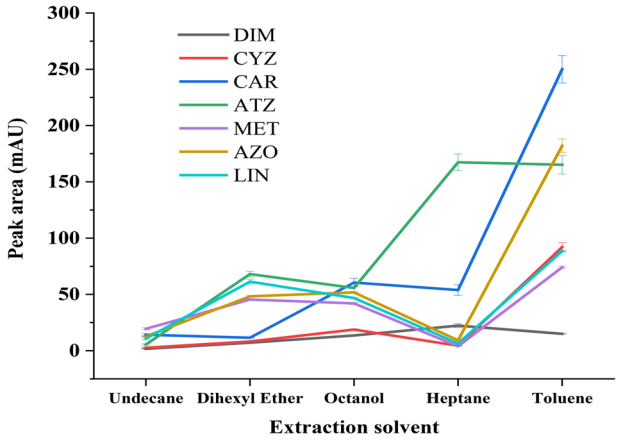


FIGURE 2 Effect of the extraction solvent type; Experimental conditions: sample volume, 5 ml; spiked concentration, 0.5 mg/L; dispersive solvent, 1000 µl ACN; sample solution pH, 5; amount of NaCl, 15% (w/v); extraction time, 2 min; centrifuging time, 5 min at 4000 rpm.

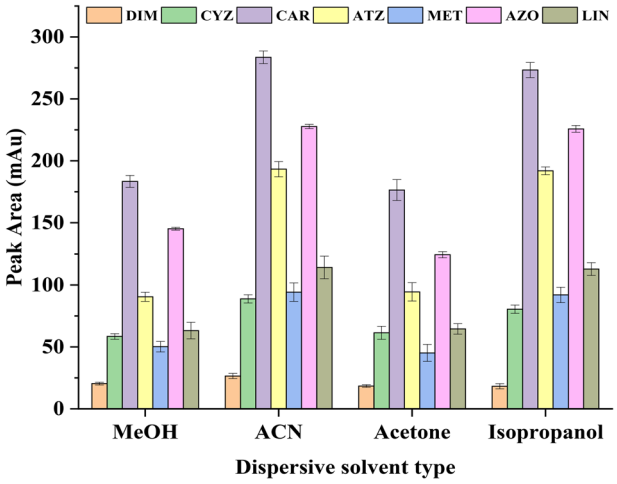


FIGURE 3 Effect of the dispersive solvent type; Experimental conditions: same as those indicated in Figure 2.

pesticides in one of the earlier studies reported in the literature [7].

3.1.2 | Extraction solvent volume

The effect of extraction solvent volume on the extraction performance of the presented DLLME–SFOD procedure was investigated by varying the volume of toluene over the range between 50 and 200 µl, with a 25 µl interval, keeping all the other experimental conditions constant. Figure S1; given in supplementary material, illustrates the peak areas versus the volume of toluene. It was observed that in the studied range, the highest peak areas were obtained for 100 µl. Therefore, this volume was chosen as optimum for all the subsequent experiments.

3.1.3 | Effect of the dispersive solvents

The dispersant allows the extractant to form suspension droplets in the aqueous phase with a large contact area in the DLLME procedure [10, 12]. The dispersive solvent must be miscible both in the aqueous phase and organic solvent. In the current study, MeOH, ACN, acetone and isopropanol were tested as dispersive solvents. During optimization, 1 ml of each of the dispersive solvent chosen was introduced into the 100 µl of extraction solvent (toluene). Similar set of dispersive solvents were also employed for quantitative extraction of other pesticides from fruit juices and the results obtained for ACN and isopropanol were closer to each other [12], which was also similar with the results obtained for the current study. However, the results obtained for this study revealed fairly better peak areas in most cases for ACN, Figure 3, and thus ACN was chosen as a preferred dispersive solvent. In other earlier studies in the fruit juice samples, for other trace pesticide residues, comparable results were also obtained and reported in the literature [16, 41].

3.1.4 | Volume of the dispersive solvent

The dispersive solvent volume directly affects the formation of the cloudy solution; the degree of dispersion of the extracting solvent in the aqueous phase and consequently the extraction efficiency. The dispersive solvent volumes most commonly employed in the DLLME are between 400 and 1400 µl [1]. In order to attain the optimal volume of the dispersive solvent, various volumes of ACN (in the range of 400–1400 µl), with 200 µl volume interval increment, were investigated. Compared with other volumes tested, for volumes less than 400 µl a stable dispersion was not formed. As shown in the data acquired from these experiments in Figure S2 (given in supplementary material), the peak area increases with the volume of ACN up to 1000 µl and then remained almost constant for 1000 and 1200 µl; which was gradually lowered with a further increase in volume. The most probable reason could be associated with the solvent volume, that is, at lower volumes the cloudy solution is not well-formed whereas, at significantly high dispersive solvent, the increased solvent volumes made the extraction solvent partly dissolved (even completely dissolved) in water, resulting in lowering the partitioning of the analytes providing low peak area [22]. Based on the experimental results, 1000 µl ACN was chosen as the optimum dispersive solvent volume.

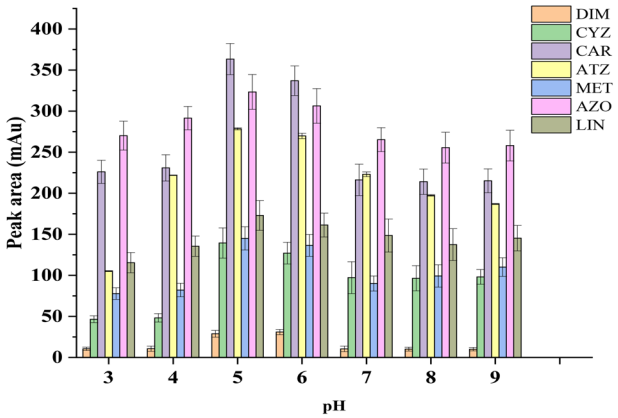


FIGURE 4 Effect of the sample solution pH; Experimental conditions: same as those indicated in Figure 3.

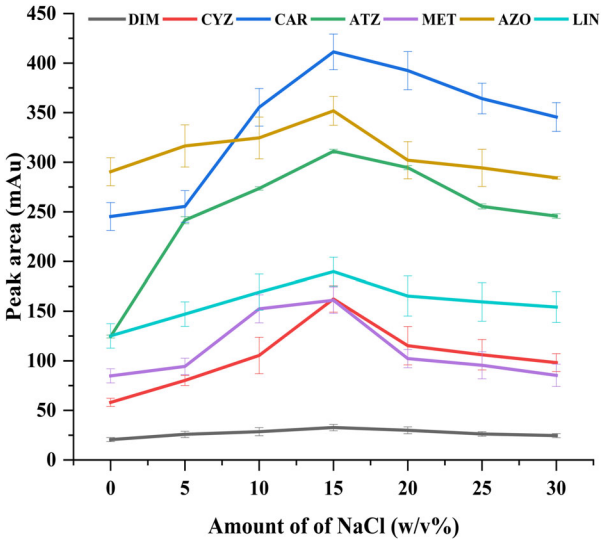


FIGURE 5 Effect of the ionic strength; Experimental conditions are the same as those indicated in Figure 4.

3.1.5 | Effect of the sample pH

The sample solution pH is the most important variable in the extraction of targeted compounds since it enables them to be identified in the uncharged state so as to easily dissolve in the organic phase rather than the aqueous phase [4, 42, 43]. To investigate the influence of pH on extraction efficiency, the pH of the sample solutions was tested in the range of 3 and 9, adjusted by 0.1 M HCl or/and 0.1 M NaOH solution, before being subjected to DLLME−SFOD. The pH range was selected based on the pK_a of the target analytes (Figure 1). It was observed that the peaks of all the target analytes were increased with an increase in pH up to 5 and then lowered at higher pH values (Figure 4). This effect may be explained by the fact that the stability of the target analytes is better enhanced in weakly acidic and weakly alkaline solutions over strongly acidic and strongly alkaline solutions, where they could readily be ionized [3]. Thus, pH 5 was selected as the optimum value for the subsequent experiments.

3.1.6 | Effect of ionic strength

The most commonly used salt for salting out pesticide residues from contaminated samples is NaCl [2, 4]. In this study, the same salt was also utilized in the concentration range from 0.5 to 30% w/v at the interval of 5% and was tested to see the effect of salt on the performance of the DLLME-SFOD procedure. It was found that increased salt concentration to 15% w/v leads to the increase in peak areas for all the target analytes except for dimethoate where the peak areas observed were constant at all concentration levels. This salting-out effect may be because there are insufficient water molecules available to dissolve the analytes due to the formation of hydration spheres by water molecules surrounding the molecules of ionic salts [4]. As can be seen in Figure 5, decreasing in the peak areas occurred when the salt contents of the sample solution increased above 15% w/v. This declining tendency might be explained by the challenge of mass transfer of the analyte from the solution into the extractant solvent. This observation could be associated with the increased viscosity of the solution upon further addition of the salt [19].

3.1.7 | The influence of vortex time on the extraction

The major function of vortex agitation is to hasten the creation of turbid toluene solution. In this study the efficiency of extraction as a function of vortex time was investigated, which ranged from 0.25 to 3 min, maintaining a constant vortex rotation speed. It took 2 min to obtain the highest vortex extraction efficiency. The rapid achievement of equilibrium could be associated with the high contact interface between the extractant and the aqueous sample [21]. There was no substantial difference as the vortex time was increased further. The data acquired from these experiments were shown in Figure S3 (supporting information); as a result, a vortex time of 2 min was chosen as the optimum extraction time.

3.2 | Analytical performance of the method

The DLLME-SFOD technique was validated in terms of linearity, LOD, LOQ, inter-day, and intra-day precision

TABLE 1 Figures of merit for the analytical performance of the proposed method.

Analyte	Linear range (ng/ml)	Regression equation	LOD[a]	LOQ[b]	r^2	Repeatability (RSD %, $n = 6$)		Reproducibility (RSD%, $n = 12$)	
						Level 1	Level 2	Level 1	Level 2
DIM	9–1500	y = 9.342x + 0.2238	0.024	0.081	0.9982 (9)[c]	2.52	3.81	5.59	5.61
CYZ	3–750	y = 159.33x + 0.2835	0.030	0.091	0.9994 (10)	5.69	4.33	6.4	3.74
CAR	3–750	y = 311.61x + 0.7714	0.021	0.070	0.9991 (10)	5.65	1.89	9.73	4.87
ATZ	3–1500	y = 237.08x – 0.4271	0.033	0.098	0.9992 (9)	5.52	1.84	6.09	3.31
MET	9–900	y = 24.786x + 0.0929	0.013	0.042	0.9993 (9)	4.09	7.42	3.94	10.78
AZO	6–1000	y = 97.983x – 0.3127	0.027	0.083	0.999 (9)	4.07	3.34	5.91	6.49
LIN	6–1000	y = 48.603x – 0.3674	0.053	0.180	0.9992 (9)	3.66	2.63	5.07	3.39

[a]Limit of detection (ng/ml); [b]Limit of quantification (ng/ml).

[c]Numbers in brackets indicate the number of concentration points for calibration curves that were prepared.

Level 1 (μg/L), 20 for ATZ, CYZ, and CAR; 40 for AZO and LIN; and 60 for DIM and MET.

Level 2 (μg/L), 80 for ATZ, CYZ, and CAR; 160 for AZO and LIN; and 240 for DIM and MET.

under optimal conditions. For ATZ, AZO, LIN, MET, and DIM, calibration curves were constructed at nine levels; for CYZ and CAR at ten levels. The linear calibration plots were generated by plotting the area of the analyte peak against the standard concentration (ng/ml). As can be seen in Table 1, for the target multiclass pesticides, linearity was expressed with correlation coefficients (r^2) ranging from 0.998 to 0.999. LOD and LOQ were computed using the following equations: $\frac{3\sigma}{m}$ and $\frac{10\sigma}{m}$, respectively, from the standard deviation (σ) of the nine blank signal analyses of reagent water and slope (m) of the working curve [42]. Hence, the LODs and LOQs were obtained in the range of 0.021–0.053 and 0.042–0.180 ng/ml, respectively.

The precision of the proposed method was evaluated in terms of repeatability (intra-day precision) and reproducibility (inter-day precision). To study the repeatability of the method, apple fruit juice sample spiked at two different concentration levels (Level 1 [μg/L], 20 for ATZ, CYZ, and CAR; 40 for AZO and LIN, and 60 for DIM and MET; Level 2 [μg/L], 80 for ATZ, CYZ and CAR; 160 for AZO and LIN; and 240 for DIM and MET) for the mixture of the seven pesticides which were extracted in duplicate. The extract of each concentration level was injected in triplicate on the same day under the optimized experimental conditions. Reproducibility of the method was also validated using the same fruit juice sample and concentration levels used above to evaluate repeatability for four consecutive days; following single extraction and triple injection. As shown in Table 1, the RSD% of the method was between 1.84 and 7.42 for intra-day and 3.74 and 10.78 for inter-day; both results of the precision studies demonstrated good reliability of the proposed analytical method.

3.3 | Applications of DLLME–SFOD to real fruit juice analyses

Five different types of fruit juices including the apple, grape, orange, pineapple, and guava samples were utilized for the accuracy validation of the proposed method. In all cases, the unspiked sample was analyzed to check the presence/absence of the seven chosen target analytes, and none of the studied fruit juice samples gave signals corresponding to concentrations above the LODs. The observed results indicated either the samples analyzed were free from the residues or contained concentrations below the detection limits. The trueness of the developed DLLME–SFOD method was evaluated from the average relative recovery of each fruit juice sample spiked at three concentration levels (Table 2). Each concentration level was extracted in triplicate. The analysis of Certified Reference Materials (CRMs) is the preferred technique to verify the method's correctness. Unfortunately, the CRM was not available in our laboratories. Thus, in order to assess the method's accuracy, relative recovery was employed by the added-found technique [39, 44, 45]. Mean relative recoveries (%RR) at three concentration levels were in the range of 87.23%–99.45%, with a %RSD of 1.37–8.39 in all kinds of fruit juice samples studied. The results obtained for recovery studies were in the acceptable range [46] indicating that the matrices of different real samples have no significant effect on the performance of the proposed method. Similar results were also reported by other workers, both for accuracy and precision analysis of pesticides in fruit juices [12, 20].

The chromatograms of the target multiclass pesticide residues in the apple fruit juice sample before and after spiking at concentration Level 2 (used for precision and

TABLE 2 The %RR and %RSD ($n = 3$) of the fruit juice samples analyzed for multiclass pesticides by DLLME-SFOD method.

Pesticides	Spiked con. (ng/ml)	Apple		Pine apple		Orange		Guava		Grape	
		RR	RSD	RR	RSD	RR	RSD	RR	RSD	RR	RSD
DIM	Level 1	95.76	4.49	96.12	5.10	98.08	2.52	92.21	3.78	89.40	3.90
	Level 2	91.45	5.99	98.24	4.05	94.51	3.81	88.65	2.04	91.13	1.98
	Level 3	99.59	3.82	89.32	7.08	95.65	2.54	93.25	3.69	87.55	3.94
CYZ	Level 1	92.45	3.93	94.57	6.10	89.42	5.69	91.23	7.29	92.03	7.22
	Level 2	96.31	2.67	93.59	1.45	98.45	4.33	95.45	6.84	87.23	5.96
	Level 3	94.49	2.36	95.12	2.38	96.24	3.23	89.54	4.88	94.25	3.73
CAR	Level 1	94.82	4.18	95.45	6.50	96.34	5.65	95.12	6.43	93.65	5.90
	Level 2	98.56	2.39	96.23	2.37	97.43	1.89	92.36	2.37	94.04	4.93
	Level 3	99.45	3.70	95.15	1.99	96.36	2.80	91.24	5.95	91.35	3.45
ATZ	Level 1	96.49	1.56	98.31	2.99	92.79	5.52	96.12	4.81	92.23	3.93
	Level 2	98.54	4.87	90.57	4.97	96.83	1.84	91.23	1.95	96.72	5.44
	Level 3	88.23	1.37	92.13	1.90	94.58	3.28	93.35	2.28	95.45	2.57
MET	Level 1	95.67	4.49	93.08	3.43	93.82	4.09	87.78	6.96	89.50	7.21
	Level 2	91.24	6.46	95.21	6.77	92.15	7.42	91.70	4.94	91.11	5.93
	Level 3	89.59	4.25	92.35	2.56	96.56	2.60	93.22	5.87	94.62	3.39
AZO	Level 1	94.45	3.84	91.54	5.19	99.24	4.07	96.25	6.76	96.43	4.67
	Level 2	96.13	2.67	90.65	2.79	97.54	3.34	92.45	4.71	87.45	7.22
	Level 3	93.48	2.39	92.23	6.40	98.13	2.17	93.89	1.85	92.35	5.85
LIN	Level 1	89.45	4.35	92.35	5.73	93.53	3.67	87.29	2.86	93.25	4.92
	Level 2	93.12	3.90	93.23	2.77	95.33	2.63	91.15	4.15	91.14	6.96
	Level 3	94.23	4.10	92.41	1.67	97.45	3.26	93.47	7.04	92.98	8.39

Level 1 (μg L^{-1}), 20 for ATZ, CYZ, and CAR; 40 for AZO and LIN; and 80 for DIM and MET.
Level 2 (μg L^{-1}), 80 for ATZ, CYZ, and CAR; 160 for AZO and LIN; and 300 for DIM and MET.

accuracy studies) using the developed DLLME–SFOD method are shown in Figure 6. The chromatograms of the target pesticides for other fruit juice samples are presented in the supporting information, Figure S4; given in the supplementary materials. It is evident from these figures that the analytes have no interference from coextracted components and are well-resolved for most of them demonstrating a high level of selectivity at their respective retention times. Therefore, the reported method is highly selective for the analyses of these and other similar multiclass pesticides in real fruit juice samples.

3.4 | Comparison of the DLLME–SFOD method with reported DLLME methods

The methods reported in the literature for analyzing pesticides in samples of fruit juice were compared in Table 3 in order to evaluate the proposed method. The presented analytical method, that is, DLLME–SFOD is more ecofriendly as an extraction organic solvent, toluene has significantly lower toxicity than those methods using toxic halogenated organic solvents [10, 11, 13, 18, 20]. Furthermore, these methods employ a microsyringe to collect the high-density microvolume of the sedimented organic layer, thus the methods are prone to more challenges compared to the DLLME–SFOD. In addition, they may be facing the problems of both sample losses and contamination during extract collection. When the present method is compared with the surfactant-emulsified vortex–assisted DLLME and magnetic dispersive SPE combined with DLLME methods [11, 19], the developed technique is found to use less solvent volume, simpler equipment, less extraction time, and so forth. Furthermore, the proposed approach exhibits a wider linear range, comparable or lower detection limit, and good precision compared to a deep eutectic solvent–based ultrasonic aided liquid-liquid microextraction method [19]. The proposed technique also offers comparable precision and improved sensitivity than majority of the reported techniques in the literature.

As a result, for enriching and selective extraction of multiclass pesticides from a variety of complex matrices, such as fruit juice samples, the method proposed in this study is a reliable alternative to the conventional DLLME methods.

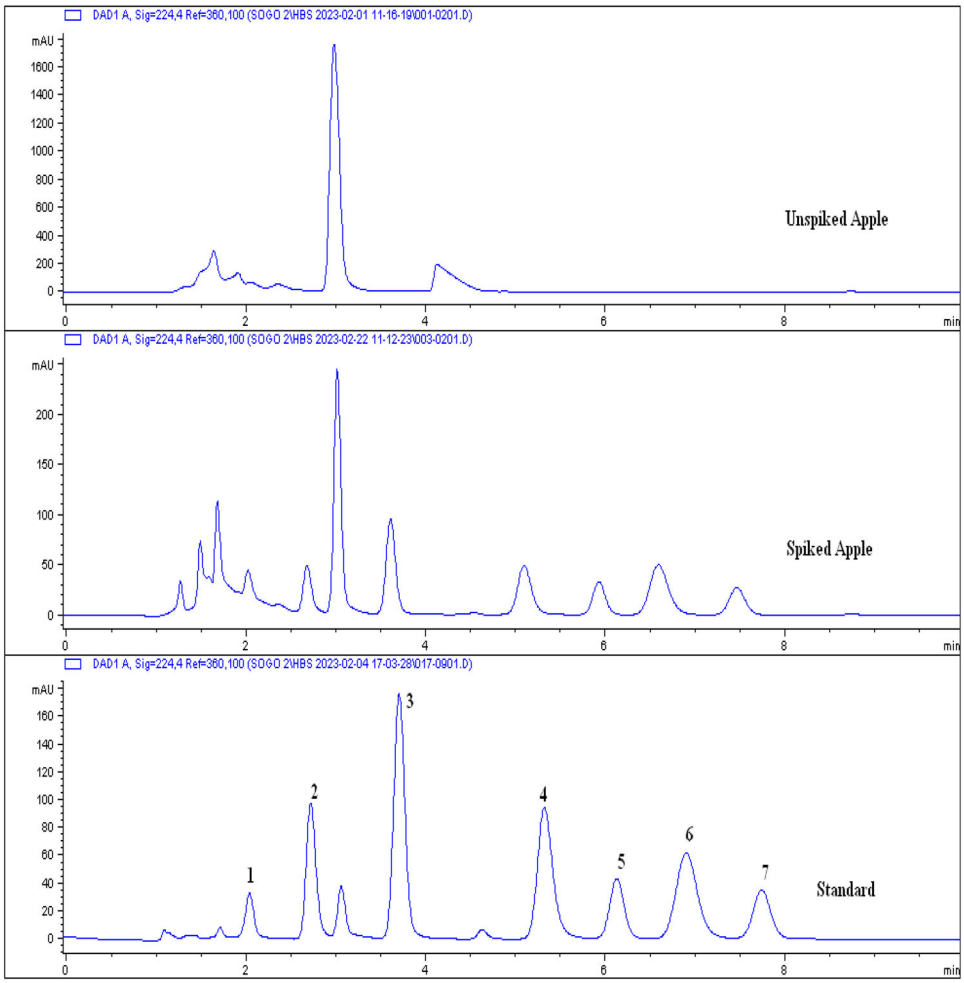


FIGURE 6 Typical chromatograms of unspiked and spiked apple juice samples at level 2; Extraction conditions: the same as those indicated in Figure 5; Peaks identifications: 1, dimethoate 2, cyanazine 3, carbaryl 4, atrazine 5, metadiothate 6, azoxystrobin 7, linuron.

TABLE 3 Comparison of the proposed method with other methods applied for the extraction and determination of pesticides in fruit juice samples.

Method	Extraction solvent	Detection	LR (μg/L)	LOD (μg/L)	RSD	Ref
MGO-SPE-DLLME	chloroform	GC-FID	3.5–10 000	1–6	4–8	[10]
MDSPE-DLLME	trichloroethane	GC-FID	0.49–5000	0.15–0.36	2–6	[11]
QuEChERS-DLLME	Carbon tetrachloride	GC—ECD	0.5–10 000	0.2–2	2.1–6.4	[13]
VA-DLLME	1-dodecanol	HPLC-DAD	20–13 590	5.58–17.40	1.2–9.8	[16]
DLLME	chloroform	HPLC—UV	2–1500	2–5	0.42–5.57	[18]
DES-UADLLME	Deep eutectic solvent	HPLC—UV	1–500	0.07–0.096	5.1–7.8	[19]
SE-VA-DLLME	Dichloromethane	LC—DAD	10–3000	0.45–0.81	3.8–7.1	[20]
VA-IL-DLLME	Ionic liquid	HPLC-DAD	10–15 000	5–10	1–3.1	[41]
DLLME-SFOD	Toluene	HPLC-DAD	3–1500	0.021–0.053	1.84–10.78	This work

Surfactant emulsified vortex assisted– DLLME (SE–VA–DLLME).
A deep eutectic solvent-based ultrasound assisted liquid-liquid microextraction (DES–UALLME).
Vortex-assisted ionic liquid–DLLME (VA–IL–DLLME).
Magnetic dispersive solid phase extraction– DLLME (MDSPE–DLLME).
Magnetic graphene oxide based–SPE–DLLME (MGO–SPE–DLLME).

4 | CONCLUDING REMARKS

In the present study, a DLLME–SFOD analytical method coupled with HPLC–DAD instrumentation was developed and optimized for selective and simultaneous sample extraction and quantitative determination of the most commonly used seven multiclass pesticides at trace levels in fruit juice samples. The extraction technique was optimized for all the experimental parameters considered in the study. During the method applied to the extraction of the trace level pesticides from fruit juice samples such as orange, grape, guava, apple, and pineapple, no matrix interferences were co-extracted and observed in the analysis at their respective retention times. The extraction solvents used in the current extraction method are more eco-friendly compared to other extraction solvents utilized in other reported studies that used toxic halogenated organic solvents. In the optimized experimental procedure, it was realized that only a low amount of extraction and disperser solvents can be used and also short equilibrium time was required to extract the analytes from samples. The suggested technique combines the advantages of a quick analysis time, simplicity, minimal organic solvent consumption, sensitivity, and cost-effectiveness as well as a high level of linearity over a wide range of analyte concentrations. Therefore, the trace level enrichment of multiclass pesticides using the DLLME–SFOD analytical technique could be considered a good alternative for selective and sensitive extraction and convenient assessment of multiclass pesticide residues in fruit juice samples in regular laboratory analysis.

AUTHOR CONTRIBUTIONS

Habtamu Bekele: Paper concept and design, sample preparation, investigation, data analysis and interpretation, and writing the original draft. Negussie Megersa: Conceptualization, data interpretation, supervision, writing review, and editing. Both authors commented on previous versions of the manuscript, and read and approved the final manuscript.

ACKNOWLEDGMENTS

The authors are thankful to the Department of Chemistry of Addis Ababa University (AAU), for the laboratory facilities. Material supports from the International Science Program (ISP) through the previous project entitled "Trace Level Pollutants Analyses (ETH:04)" is greatly acknowledged. Habtamu Bekele thanks Oromia Regional State Education Bureau for sponsoring his PhD studies.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Farajzadeh MA, Sohrabi H, Mohebbi A. Combination of modified QuEChERS extraction method and dispersive liquid –liquid microextraction as an efficient sample preparation approach for extraction and preconcentration of pesticides from fruit and vegetable samples. Food Anal Methods. 2019;12:534–43.
2. Szarka A, Búčiková K, Kostić I, Hrouzková S. Development of a multiresidue QuEChERS – DLLME—fast GC–MS method for determination of selected pesticides in yogurt samples. Food Anal Methods. 2020;13:1829–41.
3. Tolcha T, Megersa N. High density solvent based dispersive liquid–liquid microextraction technique for simultaneous and selective extraction of multiclass pesticide residues in water and sugarcane juice samples. Am J Anal Chem. 2018; 224–44.
4. Bernardi G, Kemmerich M, Machado FF, Prestes OD, Adaime MB, Zanella R. Multiresidue determination of fungicides in wine by solvent demulsification–dispersive liquid–liquid microextraction and ultra–high performance liquid chromatography–tandem mass spectrometry. Food Anal Methods. 2022;15:2026–35.
5. Tolcha T, Gemechu T, Al-Hamimi S, Megersa N, Turner C. Multivariate optimization of a combined static and dynamic supercritical fluid extraction method for trace analysis of pesticides pollutants in organic honey. J Sep Sci. 2021;44:1716–26.
6. Nemati M, Farajzadeh MA, Mohebbi A, Khodadadeian F, Afshar Mogaddam MR. Development of a stir bar sorptive extraction method coupled to solidification of floating droplets dispersive liquid–liquid microextraction based on deep eutectic solvents for the extraction of acidic pesticides from tomato samples. J Sep Sci. 2020;43:1119–27.
7. Tolcha T, Gomoro K, Megersa N. SALLE combined with LD–DLLME for pesticides analysis in sugar and soil samples. Bull Chem Soc Ethiop. 2021;35:1–16.
8. Carvalho RRR, Rodriguez MDVR, Franco ES, Beltrame F, Pereira AL, Santos VS, Araujo W, Rocha BA, Rodrigues JL. DLLME–SFO–GC–MS procedure for the determination of 10 organochlorine pesticides in water and remediation using magnetite nanoparticles. Environ Sci Pollut Res. 2020;27:45336–48.
9. Rai S, Singh AK, Srivastava A, Yadav S, Siddiqui MH, Mudiam MKR. Comparative evaluation of QuEChERS method coupled to dispersive liquid–liquid microextraction for the analysis of multiresidue pesticides in vegetables and fruits by gas chromatography–mass spectrometry. Food Anal Methods. 2016;9:2656–69.
10. Feriduni B, Mohebbi A, Farajzadeh MA, Namvar M. Magnetic graphene oxide–based solid–phase extraction combined with dispersive liquid–liquid microextraction for the simultaneous preconcentration of four typical pesticide residues in fruit juice and pulp. Food Anal Methods. 2019;12:2742–52.

11. Farajzadeh MA, Mohebbi A. Development of magnetic dispersive solid phase extraction using toner powder as an efficient and economic sorbent in combination with dispersive liquid–liquid microextraction for extraction of some widely used pesticides in fruit juices. J Chromatogr A. 2018;1532:10–9.
12. Farajzadeh MA, Kiavar L, Pezhhanfar S. Development of a method based on dispersive liquid –liquid microextraction followed by partial vaporization of the extract for ultra–preconcentration of some pesticide residues in fruit juices. J Chromatogr A. 2021;1653:462427.
13. Zhang Y, Zhang X, Jiao B. Determination of ten pyrethroids in various fruit juices: Comparison of dispersive liquid–liquid microextraction sample preparation and QuEChERS method combined with dispersive liquid–liquid microextraction. Food Chem. 2014;159:367–73.
14. Rashidipour M, Heydari R, Maleki A, Mohammadi E, Davari B. Salt-assisted liquid–liquid extraction coupled with reversed-phase dispersive liquid–liquid microextraction for sensitive HPLC determination of paraquat in environmental and food samples. J Food Meas Charact 2019;13:269–76.
15. Wang K, Xie X, Zhang Y, Pastor-Belda M, Garrido I, Campillo N, Viñas P, Hellín P, Flores P, Fenoll J. Combination of microwave-assisted extraction and ultrasonic-assisted dispersive liquid–liquid microextraction for separation and enrichment of pyrethroids pesidues in Litchi fruit prior to HPLC determination. Food Chem. 2017;233:69–76.
16. Chen Z, Li Q, Yang T, Qi L, Zhang Y, He M, Zeng H, Mai X, Liu Y, Fan H. Sequential extraction and enrichment of pesticide residues in Longan fruit by ultrasonic-assisted aqueous two–phase extraction linked to vortex-assisted dispersive liquid–liquid microextraction prior to high performance liquid chromatography analysis. J Chromatogr A. 2020, 460929.
17. Farajzadeh AM, Ali M, Sohrabi H, Mohebbi MRA. Combination of a modified quick, easy, cheap, efficient, rugged, and safe extraction method with a deep eutectic solvent based microwave–assisted dispersive liquid–liquid microextraction: Application in extraction and preconcentration of multiclass pestic. J Sep Sci. 2019;42:1273–80.
18. Boonchiangma S, Ngeontae W, Srijaranai S. Determination of six pyrethroid insecticides in fruit juice samples using dispersive liquid–liquid microextraction combined with high performance liquid chromatography. Talanta 2012;88:209–15.
19. Heidari H, Ghanbari-rad S, Habibi E. Optimization deep eutectic solvent-based ultrasound-assisted liquid–liquid microextraction by using the desirability function approach for extraction and preconcentration of organophosphorus pesticides from fruit juice samples. J Food Compost Anal. 2020;87:103389.
20. Tursen J, Yang T, Bai L, Li D, Tan R. Determination of imidacloprid and acetamiprid in bottled juice by a new DLLME–HPLC. Environ Sci Pollut Res. 2021;28:50867–77.
21. Abdallah OI, Ahmed NS. Development of a vortex-assisted dispersive liquid–liquid microextraction (VA–DLLME) and LC–MS/MS procedure for simultaneous determination of fipronil and its metabolite fipronil sulfone in tomato fruits. Food Anal Methods. 2019;12:2314–25.
22. Huang X, Du Z, Wu B, Jia L, Wang X, Jing X. Dispersive liquid–liquid microextraction based on the solidification of floating organic droplets for HPLC determination of three strobilurin fungicides in cereals. Food Addit Contam Part A. 2020;37:1279–88.
23. Zhao L, Szakas T, Churley M, Lucas D. Multi–class multi–residue analysis of pesticides in edible oils by gas chromatography–tandem mass spectrometry using liquid–liquid extraction and enhanced matrix removal lipid cartridge cleanup. J Chromatogr A. 2019;1584:1–12.
24. Zhao X, Zhou Y, Kong W, Gong B, Chena D, Weia J, Yang M. Multi-residue analysis of 26 organochlorine pesticides in Alpinia oxyphylla by GC-ECD after solid phase extraction and acid cleanup. J Chromatogr B. 2016;1017–1018:211–20.
25. Zhao W, Sun X, Deng X, Huang L, Yang M, Zhou Z. Cloud point extraction coupled with ultrasonic–assisted back–extraction for the determination of organophosphorus pesticides in concentrated fruit juice by gas chromatography with flame photometric detection. Food Chem. 2011;127:683–8.
26. Sharif Z, Man YBC, Hamid NSA, Keat CC. Determination of organochlorine and pyrethroid pesticides in fruit and vegetables using solid phase extraction clean–up cartridges. J Chromatogr A. 2006;1127:254–61.
27. Pelit FO, Pelit L, Dizdaş TN, Aftafa C, Ertaş H, Yalçınkaya EE, Türkmen H, Ertaş FN. A Novel polythiophene–ionic liquid modified clay composite solid phase microextraction fiber: preparation, characterization and application to pesticide analysis. Anal Chim Acta. 2014;859:37–45.
28. Zhao E, Han L, Jiang S, Wang Q, Zhou Z. Application of a single–drop microextraction for the analysis of organophosphorus pesticides in juice. J Chromatogr A. 2006;1114:269–73.
29. Ahmadi-Jouibari T, Pasdar Y, Pirsaheb M, Fattahi N. Continuous sample drop flow–microextraction followed by high performance liquid chromatography for determination of triazine herbicides from fruit juices. Anal Methods. 2017;9:980–5.
30. González-curbelo MÁ, Hernández-borges J, Borges-miquel TM, Rodríguez-delgado MÁ. Determination of organophosphorus pesticides and metabolites in cereal–based baby foods and wheat flour by means of ultrasound-assisted extraction and hollow-fiber liquid-phase microextraction prior to gas chromatography with nitrogenphosphorus detection. J Chromatogr A. 2013;1313:166–74.
31. Samsidar A, Siddiquee S, Shaarani SMD. A review of extraction, analytical and advanced methods for determination of pesticides in environment and foodstuffs. Trends Food Sci Technol. 2018;71:188–201.
32. Rezaee M, Assadi Y, Milani Hosseini MR, Aghaee E, Ahmadi F, Berijani S. Determination of organic compounds in water using dispersive liquid-liquid microextraction. J Chromatogr A. 2006;1116:1–9.
33. Wang XC, Shu B, Li S, Yang ZG, Qiu B. QuEChERS followed by dispersive liquid–liquid microextraction based on solidification of floating organic droplet method for organochlorine pesticides analysis in fish. Talanta 2017;162:90–7.
34. Moreno-gonzález D, Gámiz-gracia L, Bosque-sendra JM, García-campa AM. Dispersive liquid –liquid microextraction using a low density extraction solvent for the determination of 17 N–methylcarbamates by micellar electrokinetic chromatography–electrospray–mass spectrometry employing a volatile. J Chromatogr A. 2012;1247:26–34.
35. Pirsaheb M, Fattahi N, Rahimi R, Sharafi K, Ghaffari HR. Evaluation of abamectin, diazinon and chlorpyrifos pesticide residues

in apple product of Mahabad region gardens: Iran in 2014. Food Chem. 2017;231:148–55.
36. Majlesi M, Massoudinejad M Hosainzadeha F, Fattahi N. Simultaneous separation and preconcentration of phosalone and chlorpyrifos in fresh vegetables. Anal Methods. 2016;8:3795–801.
37. Pirsaheb M, Fattahi N, Pourhaghighat S, Shamsipur M, Sharafi K. Simultaneous determination of imidacloprid and diazinon in apple and pear samples using sonication and dispersive liquid–liquid microextraction. LWT-Food Sci Technol. 2015;60:825–31.
38. Sharafi K, Fattahi N, Mahvi AH, Pirsaheb M, Azizzadeh N, Noori M. Trace analysis of some organophosphorus pesticides in rice samples using ultrasound-assisted dispersive liquid– liquid microextraction and high performance liquid chromatography. J Sep Sci. 2015;38:1010–6.
39. Jouyban A, Farajzadeh MA, Mogaddam MRA. In matrix formation of deep eutectic solvent used in liquid phase extraction coupled with solidification of organic droplets dispersive liquid–liquid microextraction ; application in determination of some pesticides in milk samples. Talanta 2020, 206,120169.
40. Mao X, Wan Y, Li Z, Chen L, Lew H, Yang H. Analysis of organophosphorus and pyrethroid pesticides in organic and conventional vegetables using QuEChERS combined with dispersive liquid–liquid microextraction based on the solidification of floating organic droplet. Food Chem. 2020;309:125755.
41. Zhang Y, Zhang Y, Zhao Q, Chen W, Jiao B. Vortex-assisted ionic liquid dispersive liquid-liquid microextraction coupled with high performance liquid chromatography for the determination of triazole fungicides in fruit juices. Food Anal Methods. 2016;9:596–604.
42. Bekele H, Megersa N. Green analytical method based on salt assisted graphene oxide dispersive solid phase extraction of symmetrical triazine herbicides in environmental water samples for liquid chromatographic determination. Int J Environ Anal Chem. 2023; 1–17.
43. Ji Y, Meng Z, Zhao J, Zhao H, Zhao L. Eco–friendly ultrasonic assisted liquid–liquid microextraction method based on hydrophobic deep eutectic solvent for the determination of sulfonamides in fruit juices. J Chromatogr A. 2020;609:460520.
44. Lakew A, Assefa T, Woldeyohannes M, Megersa N, Chandravanshi BS. Development and validation of liquid chromatography method for simultaneous determination of multiclass seven antibiotic residues in chicken tissues. BMC Chem. 2022; 1–15.
45. Gure A, Lara FJ, Megersa N, Olmo-iruela M, García-campaña AM. Dispersive liquid–liquid microextraction followed by capillary high performance liquid chromatography for the determination of six sulfonylurea herbicides in fruit juices. Food Anal Methods. 2014;7:1465–73.
46. European Commission. SANTE/11813/2017 November 21–22, 2017. Guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed. 2018.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Bekele H, Megersa N. Fast surface floating organic droplets based dispersive liquid-liquid microextraction for trace enrichment of multiclass pesticide residues from different fruit juice samples followed by high performance liquid chromatography–diode array detection analysis. Sep Sci plus. 2023;e2300042. https://doi.org/10.1002/sscp.202300042

Appendix: Supplementary material

Fast surface floating organic droplets based dispersive liquid-liquid microextraction for trace enrichment of multiclass pesticide residues from different fruit juice samples followed by HPLC-DAD analysis

Habtamu Bekele, Negussie Megersa

Department of Chemistry, College of Natural and Computational Sciences, Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia

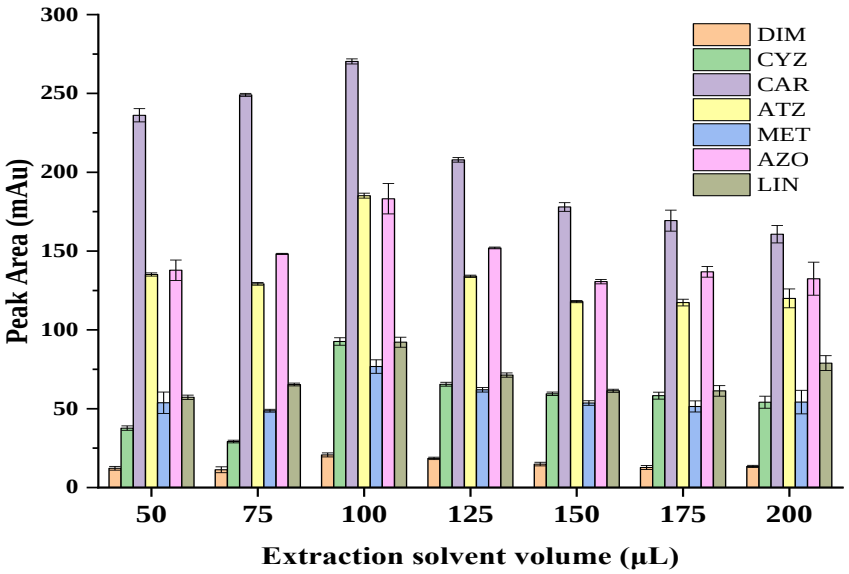


FIGURE S1 Effect of the extraction solvent volume; Experimental conditions: sample volume, 5 mL; spiked concentration, 0.5 mg/L; extraction solvent, toluene; sample pH, 5; amount of NaCl, 15% (w/v); extraction time, 2 min; centrifuging time, 5 min at 4,000 rpm.

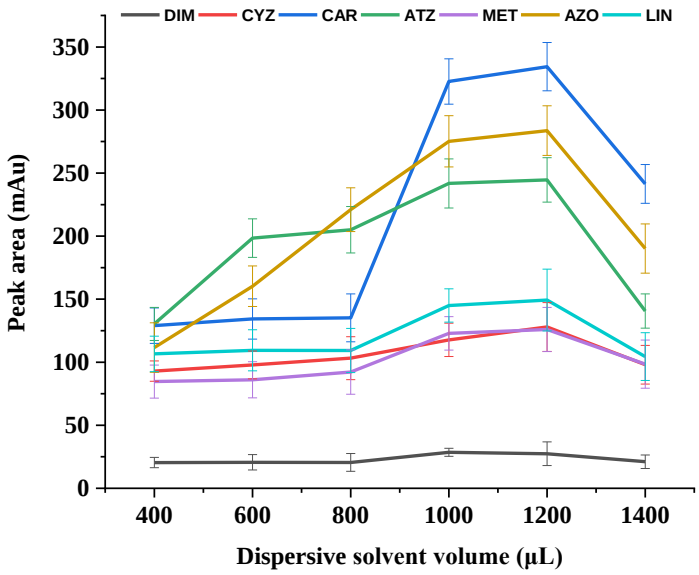


FIGURE S$_2$ Effect of the dispersive solvent volume; Experimental conditions: same as those indicated in Figure S$_1$.

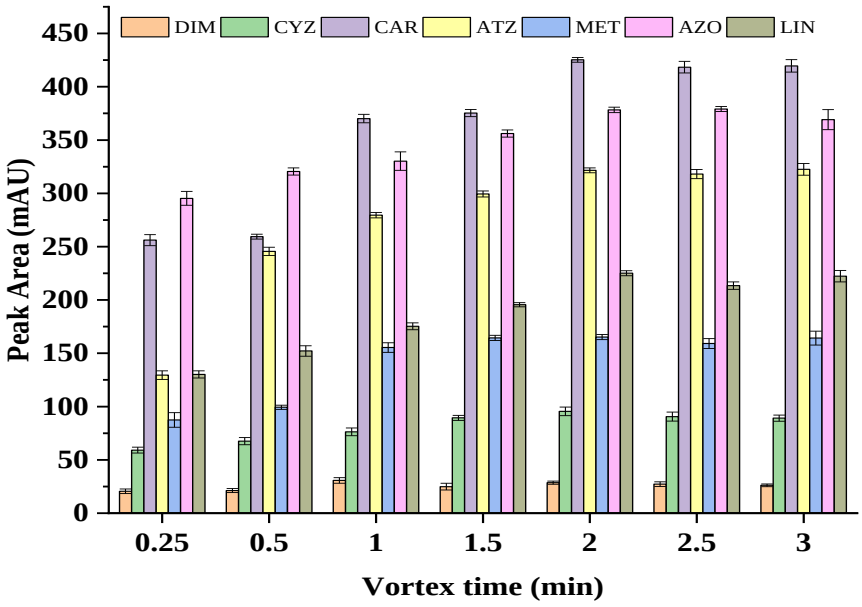


FIGURE S$_3$ Extraction time; Experimental conditions: same as those indicated in Figure S$_2$.

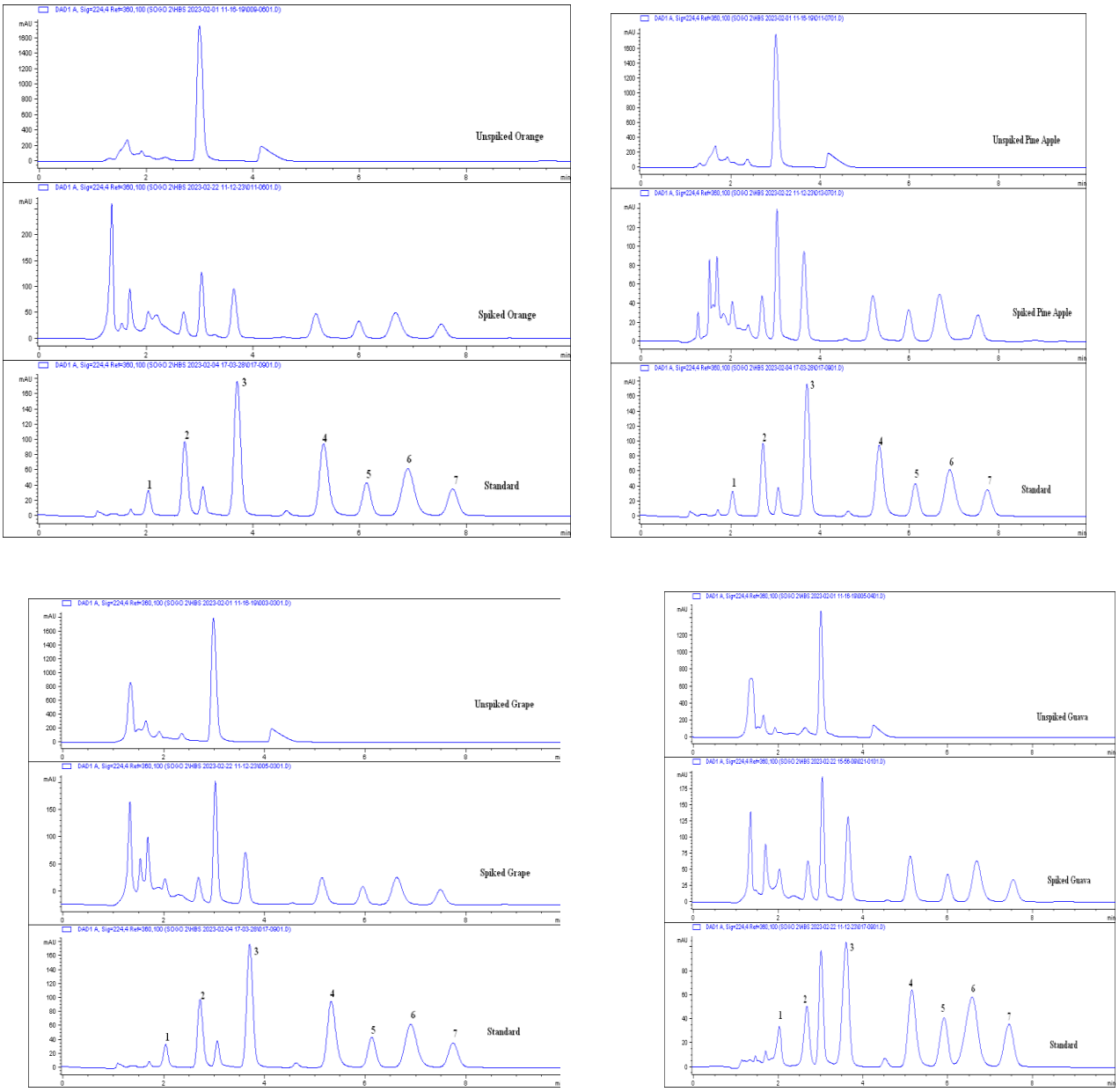


FIGURE S4 The HPLC-DAD chromatograms of blank juice samples and spiked juice samples; Experimental conditions: sample volume, 5 mL; spiked concentration, 80 μg $L^{-1}$for ATZ, CYZ and CAR; 160 μg L^{-1} for AZO and LIN; and 300 μg L^{-1} for DIM and MET; dispersive solvent, 1000 μL ACN; extraction solvent, 100 μL; sample pH, 5; amount of NaCl, 15% (w/v); extraction time, 2 min; centrifuging time, 5 min at 4,000 rpm. Peaks identification: 1, dimetathote 2, caynazine 3, carbrayl 4, atrazine 5, metadiothate 6, azoxystrobin 7, linuron.

Paper - III

(Published)

A Highly Selective Analytical Method Based on Salt Assisted Liquid-Liquid Extraction for Trace Level Enrichment of Multiclass Pesticide Residues in Cow Milk for Quantitative Liquid Chromatographic Analysis

Habtamu Bekele, Weldegebriel Yohannes, and Negussie Megersa

International Journal of Analytical Chemistry, 2023, 2023, 1-13.
https://doi.org/10.1155/2023/1754956

Hindawi
International Journal of Analytical Chemistry
Volume 2023, Article ID 1754956, 13 pages
https://doi.org/10.1155/2023/1754956

Research Article

A Highly Selective Analytical Method Based on Salt-Assisted Liquid-Liquid Extraction for Trace-Level Enrichment of Multiclass Pesticide Residues in Cow Milk for Quantitative Liquid Chromatographic Analysis

Habtamu Bekele, Weldegebriel Yohannes, and Negussie Megersa

Department of Chemistry, College of Natural and Computational Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

Correspondence should be addressed to Negussie Megersa; negussie.megersa@aau.edu.et

Received 8 June 2023; Revised 9 September 2023; Accepted 20 September 2023; Published 29 September 2023

Academic Editor: Bishnu Regmi

In this study, a simple, inexpensive, selective, and fast salting-out assisted liquid-liquid extraction (SALLE) technique coupled with high-pressure liquid chromatography-diode array detection (HPLC-DAD) was developed for the extraction, preconcentration, and analysis of trace level seven multiclass pesticide residues in pasteurized and raw cow milk samples. The significant factors that affect the extent to which the target analytes are extracted, such as the type of extraction solvent and its volume, the type and concentration of salting-out salts, the pH of the solution, and the extraction time, have been investigated. Under optimum conditions, the correlation coefficient (r^2) was obtained within a range of 0.9982–0.9997 for a broad linear range concentration of 2–1500 ng·mL^{-1}. Reliable sensitivity was achieved with limits of detection (LODs) and limits of quantification (LOQs) ranging from 0.58–2.56 ng·mL^{-1} and 1.95–8.51 ng·mL^{-1}, respectively. While precision with interday and intraday in terms of relative standard deviations (RSDs) was observed in the range of 1.97– 7.88% and 4.52– 8.04%, respectively. The results of the precision studies reveal that good repeatability and reproducibility (RSDs <9) were achieved, thus showing a low variability extraction of the developed method. Finally, the proposed and validated approach was effectively used to extract and determine pesticide residues in real milk matrices; however, the target analytes were not detected in all samples.

1. Introduction

Pesticides are chemical compounds used all over the world to control, prevent, or eliminate pests that threaten plants, animals, and human environments. It is well documented and known that their use increases agricultural productivity, though their residues greatly contaminate environmental components [1]. Nowadays, pesticide use has significantly increased throughout the world and similarly in Ethiopia [2] mainly as a result of the country's continuous agricultural reform. Out of the enormous quantities of pesticides applied, only less than 0.1% actually reach the intended pests; the remainder may end up on other environmental surfaces and accumulate in grasslands and feed additives given to cattle and other animals [3–5]. Pesticide residues at trace levels can be hazardous to unanticipated targets, posing a serious threat to human health and the ecosystem [2, 6, 7]. Humans come into contact with these chemicals through unsafe use, food, or the environment [5, 8]. Food security is a condition in which everyone, at all times, have physical, social, and economic access to sufficient quantities of wholesome foods to meet their dietary needs and food preferences for an active and healthy life [9]. Due to these facts, monitoring for pesticides in food matrices on a regular basis is crucial and has become one of the hot research topics these days [10, 11].

Pesticides typically exist in low concentrations in the environment and food matrices, and determination of these trace quantities requires various analytical instruments,

including gas chromatography-mass spectrometry (GC-MS) [7, 10, 12], gas chromatography-tandem mass spectrometry (GC-MS/MS) [13], high-performance liquid chromatography (HPLC) combined with a diode array detector (DAD) [14–16], tandem mass spectrometry (MS/MS) [1, 5, 17–19], and ultraviolet detector (UVD) [20–22]. Most of these instruments provide good selectivity, sensitivity, low detection capacity, and so on; however, there are financial limitations to acquire them at laboratories that are not well equipped to meet the demanded requirements. However, relatively less expensive techniques, such as HPLC-DAD, are routinely used for monitoring of pesticide and other pollutant residues. In addition, when combined with a DAD detection system, HPLC procedures are typically favored over GC ones since HPLC is used without derivatization and is a sufficiently selective and sensitive analytical method [23]. Therefore, HPLC-DAD was chosen for monitoring of multiclass pesticides in milk samples for the designed sample preparation methods in the current study.

Dairy farming is one of the most profitable businesses in Ethiopia, particularly in the central Oromiya regional state. Furthermore, Ethiopia has one of the highest populations of cattle in Africa, estimated at 60 million heads, and around 90% of milk products are obtained from cows [24]. Milk is one of the required food item for mankind, but the question of its contamination with trace-level pesticides must be given attention, particularly when its handling personnel are untrained farmers and agricultural extension workers who lack knowledge of pesticide management, how to use for agronomy, and veterinary care are involved [8, 25]. Studies have revealed that despite the fact that most pesticides are often present in low concentrations, their persistence causes them to accumulate in animal tissues where they enter the food chain [5, 10, 11, 19]. Contamination of milk and milk products is extremely concerning because these foods hold a very special place in the diets of infants, young children, and the elderly for whom milk is a complete diet enriched with proteins, vitamins, fats, and essential minerals [26, 27].

The health concerns posed by trace pesticide residues in food can be significant, especially for young children whose enzymatic and metabolic systems are still developing [28–30]. Research on pesticide residues in the environment and various foods that have detrimental effects on human health is receiving special attention [30]. Because milk has dissolved proteins, carbohydrates, and minerals, it is difficult to recover trace-level multiclass pesticide residues with different physicochemical properties, and thus developing an amenable sample extraction technique and cleanup step is very crucial before chromatographic analysis [26].

Among numerous sample preparation techniques that have been performed to achieve efficient extraction of pesticides from milk and milk products, liquid-liquid extraction (LLE) [13, 31], solid-phase extraction (SPE) [16], dispersive solid-phase extraction (DSPE) [19], magnetic solid-phase extraction (MSPE) [28, 32], and solid-phase microextraction (SPME) [33, 34], Quick, easy, cheap, effective, rugged, and safe (QuEChERs) [14], pressurized liquid extraction (PLE) [12], and cloud point extraction [35, 36] were some of the reported works in the literature. The majority of these methods are labor intensive, time-consuming, and environmentally unfriendly, despite the fact that they offer clear advantages for extraction of pesticides from milk. Besides, as stated explicitly in published literature, industrially produced QuEChERs kits, SPME needles, and SPE cartilage materials are quite expensive [5, 6, 37].

Preconcentration of multiclass pesticide residues in food samples nowadays needs the development of analytical techniques that are miniaturized, efficient, simple, fast, and affordable. The most popular method, dispersive liquid-liquid microextraction (DLLME), is limited to the use of nonpolar, water-immiscible solvents with low dielectric constants and poor extraction efficiency of polar organic and inorganic compounds [38]. As a result, the introduction of salt-assisted liquid-liquid extraction (SALLE), an efficient extraction method for polar to moderately polar organic compounds, was made feasible by using more polar and water-miscible organic extraction solvents like acetonitrile, isopropanol, acetone, ethanol, and methanol, among others. In the SALLE method, organic solvent is separated from the mixture, and a two-phase system is created as a result of addition of inorganic salt [39]. When using inorganic or organic salts, the salting out effect increases the ionic strength of the solution and decreases the solubility of the weak electrolyte in water, which causes the target analyte to be extracted into the organic solvent, resulting in high extraction efficiency of polar or slightly polar target analyte in an aqueous sample [39]. The SALLE method produces extracts with solutes in organic solvent that may be evaporated and reconstituted with an appropriate solvent for preconcentration and analysis by HPLC or GC [40, 41]. On the other hand, in the SALLE methods, extraction solvents are compatible with the majority of analytical instruments, particularly chromatographic ones, making it possible to directly inject the extract into these methods of analysis [36, 42, 43].

SALLE has been used successfully to analyze pesticides in foods [20, 40, 44], biological matrices [36], and environmental water [45–47]. Researchers put a lot of work into making the method automated and high throughput during the development step to reduce processing time and chemicals required [35, 37]. Though various pretreatment technologies have been developed, the method of SALLE has still been widely used, since it integrates sample cleanup, preconcentration, and extraction in one single step and shares the advantages of the sample pretreatment technique gained from QuEChERS [48, 49]. Even though numerous advantages were reported, the application of SALLE for enrichment of multiclass pesticides in milk samples is scarce in the reported literature.

To the best of our knowledge, there are no reports in the literature on the use of the SALLE technique coupled with HPLC-DAD for simultaneous extraction and determination of multiclass pesticide residues including carbamate (carbrayl), organophosphate (methidathion), triazines (cyanazine, atrazine, and propazine), neonicotinoid (thiamethoxam), and strobilurin (azoxystrobin) in cow milk samples. Therefore, the present study was designed to develop, optimize, and validate a simple, fast, inexpensive,

and an environment friendly (green) analytical technique based on SALLE, as an alternative for preconcentration and extraction of seven multiclass pesticide residues in cow milk samples.

2. Experimental

2.1. Chemicals and Reagents. The standards used in this study are of analytical reagent grade substances; methidathion was obtained from Sigma-Aldrich (St. Louis, MO, USA), and atrazine, cyanazine, and propazine were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Azoxystrobin, carbrayl, and thiamethoxam were the products of Sigma-Aldrich (Steinheim, Germany). All the pesticide standards were of the highest purity, viz., >98%. Other common chemicals used in the study were also analytical-grade reagents while the solvents utilized including acetonitrile (ACN), dihexyl ether, ethyl acetate, and acetone acquired from Sigma-Aldrich (Steinheim, Germany), methanol (MeOH) received from Carlo Erba (Rodano, Italy), and isopropanol (IPA) obtained from Sigma-Aldrich (Seelze, Germany) were HPLC-grade reagents. Magnesium sulphate anhydrous and ammonium sulphate were from Fine Chem Industries (Mumbai, India, 99%). Ammonium acetate (BDH Chemical Ltd, England, 96%) was obtained from VWR International (Radnor, PA, USA). Sodium sulphate and sodium acetate anhydrous were from (BDH Chemical Ltd, England, 96%). Common chemicals such as NaCl were obtained from Sigma-Aldrich (Steinheim, Germany), hydrochloric acid (HCl) was purchased from Sigma-Aldrich (St. Louis, MO, USA), and sodium hydroxide (NaOH) was the product of Merck chemicals (Darmstadt, Germany). Ultrapure water was prepared by purifying with a double distiller, a 8000 Aquatron water Still (Bibby Scientific, Staffordshire, UK), and a deionizer (EASY Pure LF, Dubuque).

2.2. Instruments and Equipment. Chromatographic analyses were carried out using the Agilent 1200 series HPLC system (Agilent Technologies, Waldbronn, Germany) outfitted with a quaternary pump, vacuum degasser, standard and preparative autosampler, thermostated column compartment, autosampler thermostat, and a diode array multiple wavelength detector. LC Chemstation software (B.02, 01-SR1) was used for sample processing and data acquisition. Chromatographic separation was performed using a ZORBAX ODS-C_{18} (150×3 mm, i.d., 3.5 μm particle size) analytical column from Agilent Technologies. The sample solution pH was measured using an Adwa pH meter, model 1020, made by Adwa Hungary Kft. in Szeged, Hungary. For sample preparation, a centrifuge, Model 800, Jiangsu Zhenji insturuments Co., Ltd. (Jiangsu, China), a 15 mL centrifuge tube, Corning integrated (Corning, NY, Mexico), and an ultrasonic heater, Dacon®, were utilized.

2.3. Chromatographic Conditions. Chromatographic separations were achieved using the isocratic condition of a binary mobile phase, consisting of solvent A (40% ultrapure water) and solvent B (60% methanol). Prior to the sample injection, the HPLC column was equilibrated with the mobile phase for 10 min. Analysis was performed with a flow rate of 1 mL/min, column temperature of 35°C, injection volume of 15 μL, and UV detection at 224 nm for all the target analytes. Peak area was used as instrumental response and comparison of the responses. Under these chromatographic conditions, baseline separation was maintained for all the target analytes.

2.4. Standard Solution Preparation. The stock standard solution of each target analyte, with the concentration of 0.1 mg/mL, was prepared by weighing the appropriate amount and dissolving it in methanol. Intermediate standard solutions of 10 μg/mL were obtained by diluting the stock solution with ultrapure water. Other working solutions of lower concentrations were also prepared by diluting the intermediate solution in the ultrapure water. All standard solutions were stored in the refrigerator below 4°C, when not in use. The chemical structures, common names, abbreviations, the octanol-water partition coefficient (logP; at pH 7 and 20°C), and other relevant physicochemical properties of the target pesticides considered are shown in Figure 1.

2.5. Milk Samples. A total of 7 milk samples (one fresh raw milk collected from a dairy cattle farm and three pasteurized milk processed and packed by two dairy product suppliers) were taken. Pasteurized milk samples were bought from randomly selected local supermarkets in Addis Ababa, and raw milk samples were donated from a randomly selected dairy cattle farm in Sheger city (in sululta subcity) in April 2023 for the multiclass pesticide residue analysis. After arrival at the laboratory, the pasteurized milk samples in their original packing and raw milk in a brown bottle were kept in a refrigerator at 4°C until the time of analysis, when not in use. Note that the names of the producers have been kept confidential to protect their business and reputation.

2.6. Procedure of SALLE. Aliquots of 0.5 mL of milk sample were placed in a 15 mL falcon centrifuge conical bottom tube and then diluted to 5.0 mL with ultrapure water (pH 8.0) to reduce the matrix effect of the sample. The sample solution pH was adjusted using 0.1 M HCl or 0.1 M NaOH solution and spiked with appropriate amount of mixed standard solutions of the pesticides. The sample solution was then kept to stand for 20 min to equilibrate, and 1.0 mL ACN was added and vortexed for 0.5 min. This was followed by the addition of 2.0 g $MgSO_4$ to the mixture and vortexed for an additional 2 min to dissolve the salt to be used as a salting out agent. After centrifuging the resulting content at 4000 rpm for 5 min, 500 μL of the supernatant was carefully withdrawn with a micropipette and transferred to a vial filtering through a 0.22 μL filter membrane. Then, 15.0 μL was injected into the HPLC–DAD system for extract analysis.

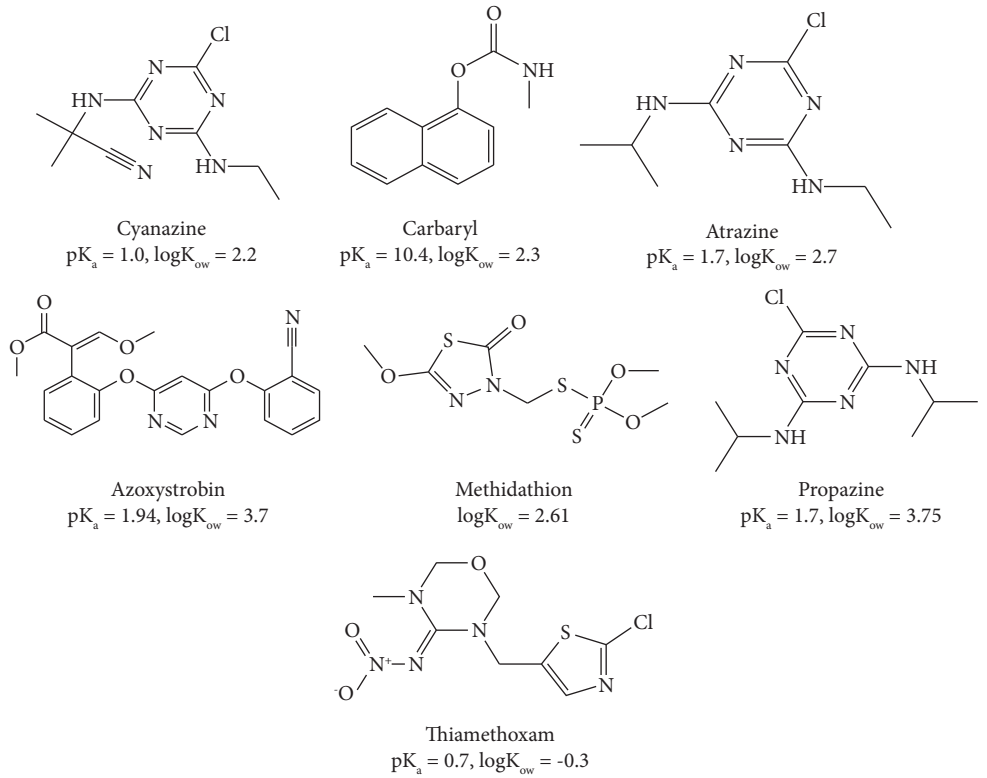


Figure 1: Chemical structure, common name, logK$_{ow}$ (octanol-water partition coefficient), and pK$_a$ (acid dissociation constant) of the multiclass pesticide compounds under study.

2.7. Statistical Analysis. Descriptive statistical analysis of means, standard deviations, and relative standard deviations for data obtained during parameter optimizations and validations of the method was performed using Microsoft Office Excel 2010 software, and figures were drawn using Origin 2019b software.

3. Results and Discussion

3.1. Optimization of the SALLE Procedure. This research work was designed with the interest of developing an efficient analytical methodology which is miniaturized, simple, fast, and cost-effective for the analysis of multiclass pesticide residues. Attainment of the desired efficiency was achieved by making use of a single sample preparation process to be able to analyze seven multiclass pesticide residues simultaneously. During method development, experiments were conducted to optimize different extraction parameters including the type and volume of the organic solvent, type and amount of salt, pH of the sample solution, and vortex time. These experimental conditions were evaluated by spiking reagent water at concentrations of 100 μg/L for CAR; 130 μg/L for THE, CYZ, ATZ, and PRZ; 260 μg/L for AZO; and 390 μg/L for MET. All the experiments were performed in triplicate (experimental) and doublet reading (instrumental). The mean peak area studies that may have impacts on the SALLE extraction efficiency were taken as instrument response when establishing the optimum experimental conditions for the following parameter under study.

3.1.1. Selection of Extraction Organic Solvent. Selection of an appropriate extraction solvent is the critical step in a SALLE procedure. The organic solvents with the desired characteristics such as high capability to dissolve the analyte, miscibility with water, ease of inducing phase separation upon addition of the appropriate salt and having good chromatographic behavior were tested as extraction solvent. Moreover, the solvent peak should not interfere with the analyte peak under the selected HPLC conditions. In this work, solvents such as MeOH, ACN, IPA, acetone, diethyl ether, and ethyl acetate were tested. A series of experiments were performed using a 5 mL ultrapure water sample containing 30% NaCl (m/v) and 2 mL of each organic solvent with the exception of methanol and acetone, in which the two phase systems were not observed. Similar observations were also noted for methanol and acetone and reported in literature by other workers [20, 22, 50]. The reason for the absence of phase separation in methanol could be due to the high polarity of methanol caused by its hydroxyl group and the hydrogen bond formed between this solvent and water which as a result increases its solubility [51]. Figure 2 depicts the observed maximum peak area when ACN was used as the extraction solvent. This might be attributed to its closer polarity with water and its promising protein precipitation reagent for milk [52]. Additional advantages of using ACN as an extraction solvent are its ability to extract a wide range of compounds [53] caused by its higher polarity and its less toxic and less harmful nature compared to other common extraction solvents. These characteristics also make

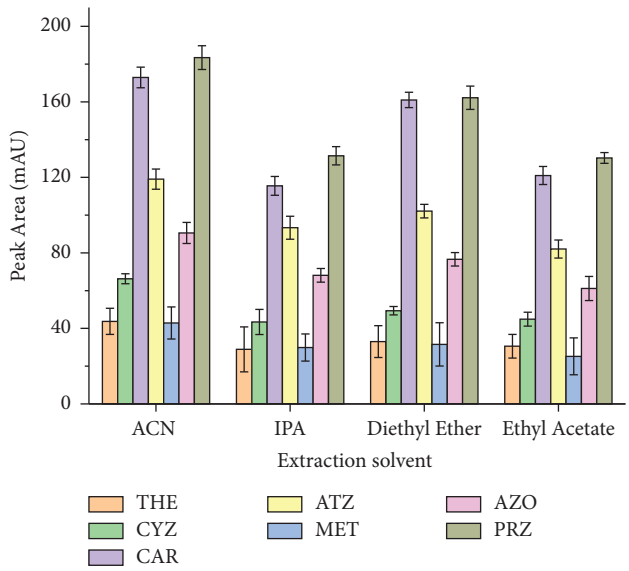


Figure 2: Effect of extraction solvent type. Extraction conditions: sample size, 5 mL; amount of salt added, 30% NaCl (m/v); pH of solution, 7.0; vortex agitation time, 1 min; volume of each extraction solvent, 2 mL; centrifugation speed, 4000 rpm for 5 min; $n = 6$.

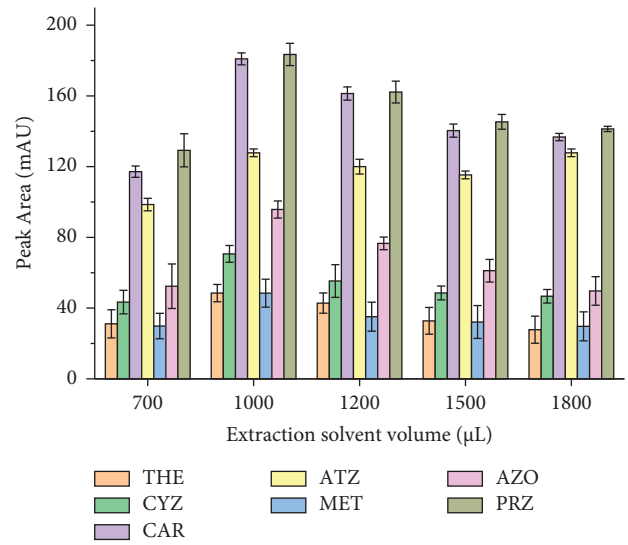


Figure 3: Effect of extraction solvent volume. Extraction conditions: sample size, 5 mL; extraction solvent, acetonitrile; amount of salt added, 30% NaCl (m/v); pH of solution, 7.0; vortex agitation time, 1 min; centrifugation speed, 4000 rpm for 5 min; $n = 6$.

it more suitable from the viewpoint of green chemistry. Thus, ACN is selected to be used as extraction solvent in this study.

3.1.2. Volume of the Extraction Solvent Effect. The volume of the extraction solvent is a very crucial parameter that influences the extraction performance of the SALLE technique since it affects the amount of analyte solubility in the sample solution [51]. Generally, the volume of extraction solvent used should be as low as possible to achieve the highest possible enrichment and the least toxicity hazards for environment. In this context, the influence of the ACN volume on the extraction efficiency was investigated between 700 and 1800 μL. As shown in Figure 3, peak areas of all the analytes increased with the volume of ACN from 700 to 1000 μL and then decreased upon further increase in the volume of the ACN. With low volumes, i.e., lower than 700 μL, the interface between the extraction solvent and the aqueous phases was not clear, and collection of the organic layer was found to be difficult. A decrease in extraction efficiency above 1000 μL may be due to the dilution effect resulting from the higher volume of the organic phase obtained after extraction, and hence further higher volumes were not performed [22]. Hence, based on the observed experimental results, 1000 μL ACN was selected as the optimum volume in all the subsequent experiments.

3.1.3. Effects of the Salt Type. The solubility of both the analytes as well as the extraction solvent in the aqueous phase could be decreased by salt addition, and this in turn enhances the analytes transfer into the organic phase [44, 48]. As different salts have the capacity to cause different degrees of phase separation [52], in this study, the effect was evaluated by addition of the salts such as NaCl, $(Na)_2SO_4$, $MgSO_4$, $(NH_4)_2SO_4$, and $\mathbf{NH_4CH_3CO_2}$, using 30% (m/v) of each salt, as potential salting out agent. It was observed that all salts could induce phase separation, but as it can be seen from Figure 4, the highest instrumental response for all analytes was obtained when $MgSO_4$ was used as the salting out agent. This could be due to its high ionic strength per unit concentration in the aqueous phase [43].

3.1.4. Effect of Salt Concentration. Varying salt concentrations may cause the degrees of phase separation to vary [43, 54]. A salting-out study was carried out by adding different amounts of $MgSO_4$ in the range of 0.75 g–2.5 g (or 15–50%, m/v) in the aqueous sample solution. It was shown that in Figure 5, the peak area of the target analytes was slightly increased as the concentration of the salt increases from 1 g to 2 g. However, at higher concentrations, the peaks were observed to slightly decrease for all the target analytes, and thus 40% m/v (2 g) was chosen to be the optimum for the following experiments. Similar quantities of this salt were found to cause a significant salting-out effect in the SALLE analytical method, reported in the literature and employed for fruit juice, yogurt, and carbonated drink matrices [41, 44].

3.1.5. Effect of Sample pH. In SALLE, the sample solution pH also has a significant role on the extraction efficiency of the multiclass pesticides, as it affects the extent of their ionization as well as the solubility in aqueous media [35, 51, 55]. The effect of this parameter was evaluated by carrying out a series of experiments varying the pH values from 3.0 to 9.0 in the aqueous solution. These pH values were adjusted using HCl and NaOH. The experimental results

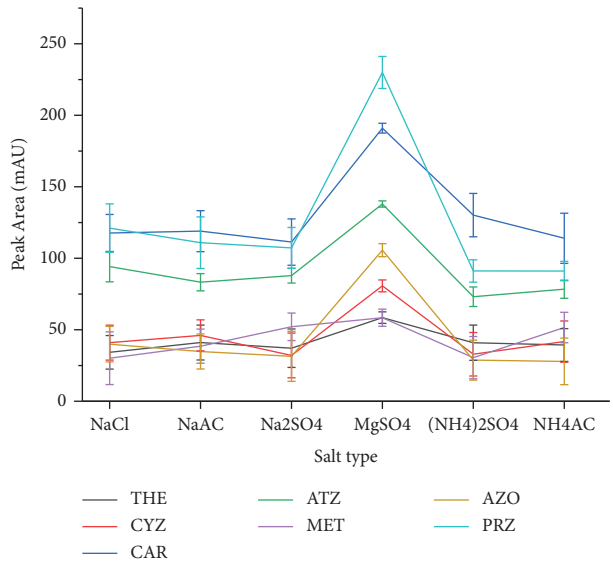


Figure 4: Effect of the salt type. Extraction conditions: sample size, 5 mL; extraction solvent, acetonitrile; extraction solvent volume; 1000 μL; amount of each salt added, 30% (m/v); pH of solution, 7.0; vortex agitation time, 1 min; centrifugation speed, 4000 rpm for 5 min; $n = 6$.

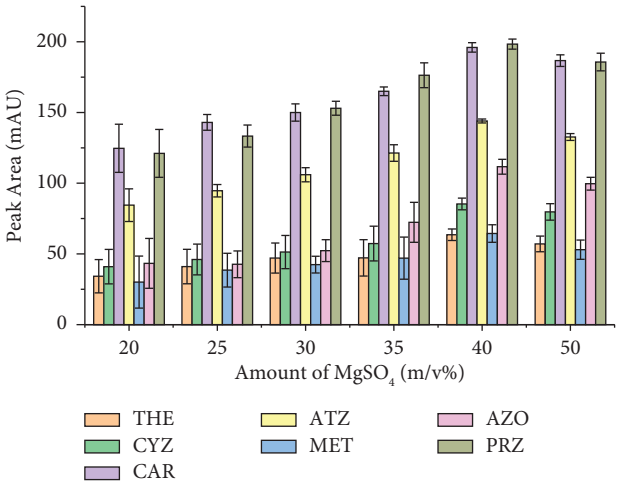


Figure 5: Effect of the amount of $MgSO_4$. Extraction conditions: sample size, 5 mL; extraction solvent, acetonitrile; extraction solvent volume, 1000 μL; salt type, $MgSO_4$; pH of solution, 7.0; vortex agitation time, 1 min; centrifugation speed, 4000 rpm for 5 min, $n = 6$.

obtained revealed that pH 8 was the optimum value, as shown in Figure 6. This could mainly be associated with the enhanced stability of the target analytes in the weakly alkaline solution, while they were easily degraded in acidic and strongly alkaline environments [4]. Therefore, pH 8 was selected as the optimum value for the subsequent studies.

3.1.6. Effect of Centrifugation Time. In SALLE procedures, optimizing the time required for phase separation is also an important analytical step, in order to obtain a clear extract [44]. In order to establish the optimum conditions:

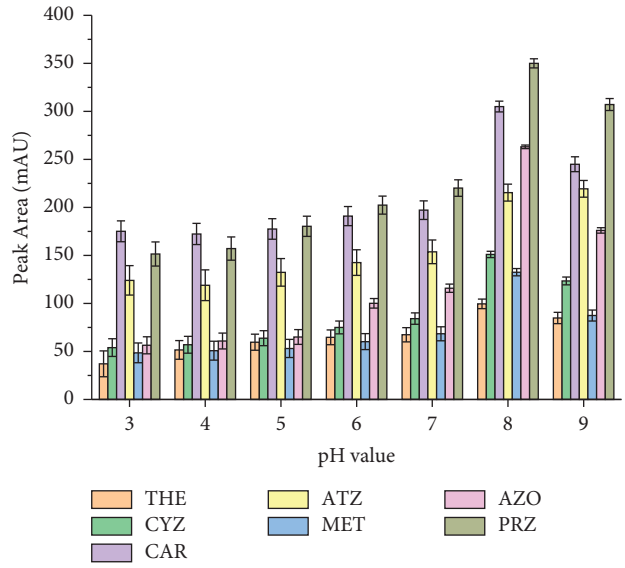


Figure 6: Effect of pH. Extraction conditions: sample size, 5 mL; extraction solvent, acetonitrile; extraction solvent volume, 1000 μL; salt type, $MgSO_4$; amount of $MgSO_4$ added, 40% (m/v); vortex agitation time, 1 min; centrifugation speed, 4000 rpm for 5 min; $n = 6$.

centrifugation time was varied between 1 and 7 min, with a 2 min interval, at a constant speed of 4000 rpm. Based on the peak areas representing the target analytes, the highest results were obtained at the centrifugation time of 5 min (shown in supplementary material Figure S1). Therefore, centrifugation time of 5 min was selected as the optimum time for the subsequent studies.

3.1.7. Effect of Vortex Agitation Time. Mass transfer is a time-dependent process and one of the most important factors in most of the extraction procedures [44]. Vortex was performed to strengthen the contact between acetonitrile and the aqueous sample solution (i.e., influence the kinetics of the extraction), thus facilitating the formation of the two-phase system. Besides, in the present study, vortex agitation was also employed to enhance the dissolution of the salting-out salt. Therefore, a vortex time was evaluated in the range of 0.25–4 min, at the maximum vortex speed, and thus a slight increase of peaks was obtained when the vortex time increased from 15 sec to 30 sec. This indicates that the diffusion of pesticides from the sample to the acetonitrile medium was found to require a short time. A decrease in extraction efficiency after 30 sec (Figure 7) may be associated to the back extraction. Thus, extraction time of 30 sec was chosen in the present study.

3.2. Analytical Performance of the Proposed Method

3.2.1. Calibration Curves and Precision Study. The proposed analytical method was validated through linearity and analytical figures of merit under optimal conditions. Linearity validation of the method was performed with the establishment of the linear calibration using external standard,

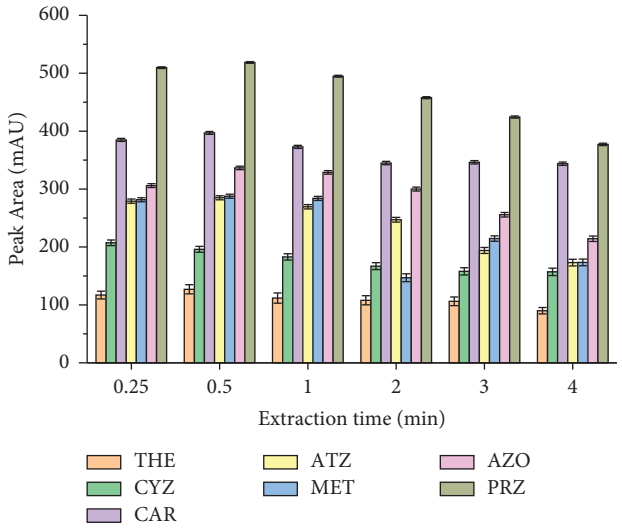


Figure 7: Effect of the extraction time. Extraction conditions: sample size, 5 mL; extraction solvent, acetonitrile; extraction solvent volume, 1000 μL; salt type, $MgSO_4$; amount of $MgSO_4$ added, 40% (m/v); pH of solution, 8.0; centrifugation speed, 4000 rpm for 5 min; $n = 6$.

and the corresponding curves (supplementary information, Figure S2) were generated by plotting the area of the analyte peak against the standard concentration (ng/mL). Good linearity, with a correlation coefficient >0.998 was obtained for all the target analytes considered in this study over the studied concentration range (Table 1). Individual chromatograms of the target analyte considered in this study are given in Figure S3.

The precision of the proposed method was also evaluated in terms of repeatability (intraday precision) and reproducibility (interday precision). To study repeatability of the method, pasteurized milk sample one (PSM) was spiked with the mixture of seven pesticides at two concentration levels ($\mu g\ L^{-1}$): Level 1 : 10 for CAR; 15 for THE, CYZ, ATZ, and PRZ; 30 for AZO; and 45 for MET, and Level 2 : 100 for CAR; 130 for THE, CYZ, ATZ, and PRZ; 260 for AZO; and 390 for MET. The sample was extracted in triplicate and injected in duplicate on the same day under the optimized experimental conditions. The reproducibility of the method was also validated using the same milk sample at concentration values used above to evaluate reproducibility for four consecutive days, following single extraction and injection. As shown in Table 1, the RSD % of the method was in the range of 1.97–7.88 for intraday and 4.52–8.04 for interday. The results of the precision studies reveal that good repeatability and reproducibility (RSD <9) were achieved, thus showing a low variability extraction technique [35, 56].

3.2.2. Sensitivity. The sensitivity of the method guaranteed the detection and confirmation of pesticide residues in milk found at levels below or above the limits of detection (LODs). The calculations for LODs and limits of quantitation (LOQs) were based on the standard deviation (σ) of the seven extraction responses of blank milk for each type of milk samples and the slope of the calibration curve (S) using equations $3 \times \sigma/S$ and $10 \times \sigma/S$, respectively [55]. The results are given in Table 1, showing that the LODs ranged from 0.58 to 2.56 $ng{\cdot}mL^{-1}$ while LOQs from 1.95 to 8.51 $ng{\cdot}mL^{-1}$.

3.3. Applications of the SALLE Method to Real Milk Samples. The suggested method's accuracy was validated using three real milk samples including pasteurized milk sample one (PMM), pasteurized milk sample two (PSM), and raw sululta milk sample (RSM). None of the tested milk samples produced signals corresponding to values above the LODs when the unspiked sample was evaluated to determine whether the seven selected target analytes were identified or not. The observed results may indicate that the samples tested were either free of pesticide residues or contained amounts below the detected limits. The average relative recovery (RR%) of each sample spiked at two concentration levels and extracted in triplicate was used to determine the accuracy of the proposed SALLE technique (Table 2). Relative recovery was intended using the standard addition on the blank real samples to evaluate the methods accuracy [21, 22, 35]. Mean relative recoveries (RR %) at two concentration levels were in the range of 85.9–108.8%, with %RSD <11.5 for the studied milk samples. The results obtained for recovery were in the acceptable range [56], indicating that the matrices of milk samples have no intense effect on the performance of the proposed method. Similar results were also reported by other workers both for accuracy and precision for the analysis of pesticides in the studied milk [35].

The chromatograms of the target multiclass pesticide residues in the PSM milk sample before and after spiking at concentration (level 2) used for precision study using the developed SALLE methods are shown in Figure 8. The separation of target analytes in the chromatogram obtained using reversed-phase high-performance liquid chromatography is in the order of their polarity in which the more polar elute first and the less polar one retained more (Figure 1, $\log K_{ow}$ value). The chromatograms selectivity was assessed by comparing blank and fortified sample peaks. It is evident from these chromatograms that absence of the chromatographic peaks from coextracted components and is well resolved for all analytes, demonstrating a high level of selectivity at the same retention time as the target pesticides. Therefore, the reported chromatogram endorses the selectivity of the developed SALLE technique. The other milk samples evaluated by this study also had the same profiles (supplementary information, Figures S4 and S5).

3.4. Comparison of the Proposed Method to Other Previously Reported Methods for the Analysis of Milk Samples. The presented analytical method, i.e., SALLE-HPLC-DAD for preconcentration and determination of multiclass pesticide residues was compared with other methods reported in the literature, such as dispersive liquid-liquid microextraction with gas chromatography mass spectrometry (DLLME-GC-MS) [17], solid-phase extraction with high performance liquid chromatography coupled with ultraviolet detector (SPE-HPLC-UV) [16], cloud point extraction with HPLC-UV (CPE-HPLC-UV) [3], head space solid-

Table 1: Analytical figures of merit for the SALLE technique combined with HPLC-DAD for multiclass pesticide residues under study.

Analyte	Linear range (ng/mL)	Regression equation	LOD (ng/mL)	LOQ (ng/mL)	r^2	[a]Repeatability (RSD %, $n = 6$)		[a]Reproducibility (RSD%, $n = 12$)	
THE	10–750	$y = 0.116x + 2.3401$	2.27	7.58	0.9993	[b]2.02	[c]3.38	[b]5.56	[c]7.77
CYZ	3–1000	$y = 0.1776x + 2.7101$	1.43	4.76	0.9997	3.54	2.05	6.57	4.52
CAR	2–750	$y = 0.3345x + 2.8145$	1.76	5.88	0.9992	5.50	5.13	7.32	5.13
ATZ	10–1000	$y = 0.2883x + 6.8847$	0.68	2.26	0.9994	3.11	1.97	8.13	6.57
MET	8–1500	$y = 0.0384x + 1.8033$	2.56	8.51	0.9991	4.40	4.04	7.52	6.65
AZO	5–1500	$y = 0.1033x + 2.7038$	2.05	6.84	0.9991	6.44	7.88	5.99	8.04
PRZ	3–1000	$y = 0.4143x + 8.486$	0.58	1.95	0.9982	2.88	5.13	7.67	7.59

[a]Validated using a pasteurized milk sample one (PSM) sample. [b]Level 1 : 10 μg/L for CAR; 15 μg/L for THE, CYZ, ATZ, and PRZ; 30 μg/L for AZO; 45 μg/L for MET. [c]Level 2 : 100 μg/L for CAR; 130 μg/L for THE, CYZ, ATZ, and PRZ; 260 μg/L for AZO; 390 μg/L for MET.

Table 2: Relative recovery (RR) values of the proposed method in the milk samples.

Sample	Spiked level	Analytes						
		THE	CYZ	CAR	ATZ	MET	AZO	PRZ
		%RR (%RSD, $n = 3$)						
PSM	Level 1	95.7 (2.6)	93.0 (4.9)	92.8 (4.3)	87.7 (7.3)	90.2 (5.5)	92.8 (6.1)	108.8 (3.3)
	Level 2	93.3 (4.2)	94.2 (3.0)	104.6 (5.5)	92.3 (8.0)	87.5 (4.8)	89.7 (5.4)	96.7 (2.7)
PMM	Level 1	97.9 (7.3)	93.2 (4.6)	91.7 (4.6)	87.9 (7.0)	91.6 (4.1)	85.9 (4.6)	108.3 (8.9)
	Level 2	92.9 (7.0)	94.3 (3.1)	97.5 (5.0)	91.3 (3.4)	90.0 (4.0)	88.5 (7.0)	92.9 (3.3)
RSM	Level 1	96.0 (7.8)	86.3 (8.9)	88.4 (11.4)	88.2 (8.4)	85.3 (10.3)	88.6 (5.2)	94.2 (11.3)
	Level 2	97.1 (10.6)	91.6 (6.2)	97.0 (8.0)	92.5 (5.1)	92.8 (9.2)	87.1 (6.2)	89.5 (10.9)

PSM, pasteurized milk sample one; PMM, pasteurized milk sample two; and RSM, raw sululta milk sample. Level 1 : 10 μg/L for CAR; 15 μg/L for THE, CYZ, ATZ, and PRZ; 30 μg/L for AZO; 45 μg/L for MET. Level 2 : 100 μg/L for CAR; 130 μg/L for THE, CYZ, ATZ, and PRZ; 260 μg/L for AZO; 390 μg/L for MET.

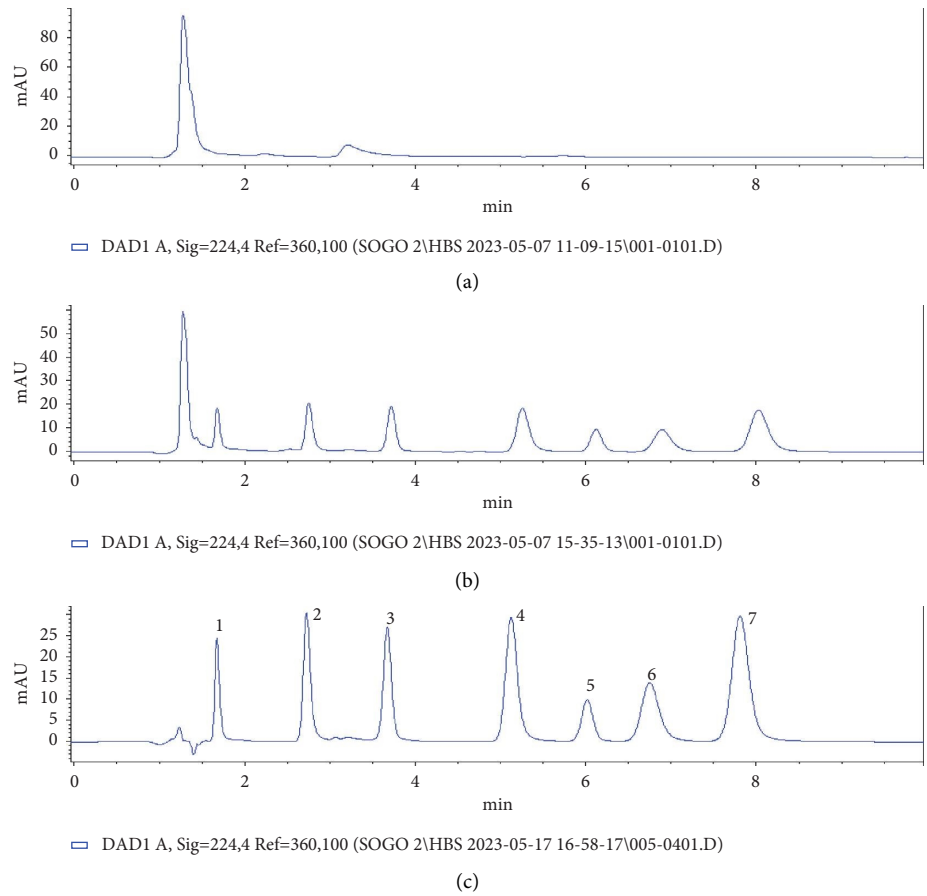


Figure 8: Typical chromatograms of blank (a), unspiked (b), and spiked (c) PSM milk samples at a concentration level 2 (100 μg/L for CAR; 130 μg/L for THE, CYZ, ATZ, and PRZ; 260 μg/L for AZO; 390 μg/L for MET). Extraction conditions: sample size, 5 mL; extraction solvent, acetonitrile; extraction solvent volume, 1000 μL; salt type, $MgSO_4$; amount of $MgSO_4$ added, 40% (m/v); extraction time, 0.5 min; pH of solution, 8.0; centrifugation speed, 4000 rpm for 5 min. Peaks identifications: 1, thiamethoxam; 2, cyanazine; 3, carbrayl; 4, atrazine; 5, methidathion; 6, azoxystrobin; 7, propazine.

phasemicroextraction with GC-MS (HS-SPME-GC-MS) [33], quick, easy, cheap, effective, rugged, and safe (QuEChERS) coupled with HPLC and diode array detector (QuEChERS-HPLC-DAD) [14], and dispersive solid-phase extraction combined DLLME with HPLC-DAD (DSPE-DLLME-HPLC-DAD) [57], and the results are shown in Table 3. As can be seen, in terms of the LODs, precisions and accuracy of the present method were better than or comparable to those of the other methods applied for extraction of pesticides from the same type of matrices, i.e., milk sample. For HS-SPME [33] and SPE [16] methods, there may be the problem of facing sample carryover effects which leads to false-positive results. The proposed SALLE is simple, and unlike the SPE method, it does not require multisteps conditioning, washing, loading, and elution [5, 6]. In addition, the proposed method is found to use simpler equipment and exhibits a wider linear range, integrated

Table 3: Comparison of the proposed method with other methods applied for the extraction and determination of pesticides in milk samples.

Methods	Detection	Extraction time (min)	LR (μg L^{-1})	LOD (μg L^{-1})	RSD	RR	Ref.
CPE	HPLC–UV	30	50–2000	6.79–11.2	1.41–5.99	70.5–96.9	[3]
QuEChERS	HPLC–DAD	1	—	20–60	1–23	35–131	[14]
SPE	HPLC–UV	20	1–320	0.12–0.40	5.1–6.3	86–110	[16]
DLLME	GC–MS	0.5	2–1000	0.9–5.0	1.02–4.18	86.15–112.45	[17]
HS-SPME	GC–MS	45	6.5–56	2.2–10.9	6.1–29.5	—	[33]
DSPE–DLLME	HPLC–DAD	11	0.57–1000	0.17–0.36	3.3–7.2	79–92	[56]
SALLE	HPLC–DAD	0.5	2–1500	0.58–2.56	1.97–8.04	85.9–108.8	This work

"—", not reported.

pretreatment and preconcentration in a single step, which would make the procedure simpler, cost-effective, time saving, and eco-friendly.

4. Conclusions

In this study, the SALLE-HPLC-DAD analytical technique, that is, simple, fast, and green, was developed and optimized for routine monitoring and quantitative determination of seven multiclass pesticide residues with a wide range of physicochemical properties including methidathion, atrazine, azoxystrobin, cyanazine, carbrayl, thiamethoxam, and propazine in samples of raw and pasteurized milk. Compared with more traditional extraction techniques like LLE and SPE, this method uses a significantly smaller extraction solvent and sample volume. For all of the experimental factors taken into account during the investigation, the approach was optimized utilizing univariate methods. The optimized method offers sufficient accuracy, precision, linearity, and sensitivity under optimum extraction conditions in a short extraction time. No matrix interferences were coextracted or seen in the analysis at their respective retention times while this method was being used to extract trace-level pesticides from milk samples. Comparatively to other reported research that used hazardous halogenated organic solvents as extraction solvents, the extraction solvents used in the current extraction approach are more environmentally benign. As a result, the trace level enrichment of multiclass pesticides using the SALLE analytical technique could be thought of as a good alternative for selective and sensitive extraction and practical assessment of multiclass pesticide residues in milk as well as enrichment of other trace compounds in complex samples in routine laboratory analysis.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors are thankful to the Department of Chemistry of the Addis Ababa University (AAU) for the laboratory facilities. Material supports from the International Science Program (ISP) through the previous project entitled "Trace Level Pollutants Analyses (ETH:04)" are greatly acknowledged. Habtamu Bekele thanks Oromia Regional State Education Bureau for sponsoring his doctoral studies.

Supplementary Materials

Figure S1: effect of centrifugation time. Extraction conditions: sample size, 5 mL; extraction solvent, acetonitrile; extraction solvent volume, 1000 μL; salt type, $MgSO_4$; amount of $MgSO_4$ added, 40% (m/v); extraction time, 0.5 min; pH of solution, 8.0; and centrifugation speed, 4000 rpm; $n = 6$. Figure S2: calibration curve of (a) thiamethoxam, (b) cyanazine, (c) carbrayl, (d) atrazine, (e) methidathion, (f) azoxystrobin, and (g) propazine. Figure S3: chromatograms of individual pesticides: (1) thiamethoxam; (2) cyanazine; (3) carbrayl; (4) atrazine; (5) methidathion; (6) azoxystrobin; and (7) propazine. Figure S4: typical chromatograms of blank (A), unspiked (B), and spiked (C) PMM milk sample at concentration level 2 (100 μg/L for CAR; 130 μg/L for THE, CYZ, ATZ, and PRZ; 260 μg/L for AZO; and 390 μg/L for MET). Extraction conditions: sample size, 5 mL; extraction solvent, acetonitrile; extraction solvent volume, 1000 μL; salt type, $MgSO_4$; amount of $MgSO_4$ added, 40% (m/v); extraction time, 0.5 min; pH of solution, 8.0; and centrifugation speed, 4000 rpm for 5 min. Peaks identifications: (1) thiamethoxam; (2) cyanazine; (3) carbrayl; (4) atrazine; (5) methidathion; (6) azoxystrobin; and (7) propazine. Figure S5: typical chromatograms of blank (A), unspiked (B), and spiked (C) RSM milk sample concentration level 2 (100 μg/L for CAR; 130 μg/L for THE, CYZ, ATZ, and PRZ; 260 μg/L for AZO; and 390 μg/L for MET). Extraction conditions: sample size, 5 mL; extraction solvent, acetonitrile; extraction solvent volume, 1000 μL; salt type, $MgSO_4$; amount of $MgSO_4$ added, 40% (m/v); extraction time, 0.5 min; pH of solution, 8.0; and centrifugation speed, 4000 rpm for 5 min. Peaks identifications: (1) thiamethoxam; (2) cyanazine; (3) carbrayl; (4) atrazine; (5) methidathion; (6) azoxystrobin; (7) propazine. (*Supplementary Materials*)

References

[1] F. Zhang, Q. Zhao, X. Yan et al., "Rapid preparation of expanded graphite by microwave irradiation for the extraction of triazine herbicides in milk samples," *Food Chemistry*, vol. 197, pp. 943–949, 2016.

[2] B. M. Teklu, P. I. Adriaanse, and P. J. Van den Brink, "Monitoring and risk assessment of pesticides in irrigation systems in Debra Zeit, Ethiopia," *Chemosphere*, vol. 161, pp. 280–291, 2016.

[3] T. Liu, P. Cao, J. Geng et al., "Determination of triazine herbicides in milk by cloud point extraction and high performance liquid chromatography," *Food Chemistry*, vol. 142, pp. 358–364, 2014.

[4] H. Bekele and N. Megersa, "Fast surface floating organic droplets based dispersive liquid-liquid microextraction for trace enrichment of multiclass pesticide residues from different fruit juice samples followed by high performance liquid chromatography–diode array detection analysis," *Separation Science Plus*, vol. 6, no. 8, pp. 1–12, 2023.

[5] L. Lachat and G. Glauser, "Development and validation of an ultra–sensitive UHPLC-MS/MS method for neonicotinoid analysis in milk," *Journal of Agricultural and Food Chemistry*, vol. 66, no. 32, pp. 8639–8646, 2018.

[6] A. Samsidar and S. Siddiquee, "A review of extraction, analytical and advanced methods for determination of pesticides in environment and foodstuffs," *Trends in Food Science & Technology*, vol. 71, pp. 188–201, 2018.

[7] A. Szarka, K. Búčiková, and I. Kostić, "Development of a multiresidue QuEChERS–DLLME–fast GC–MS method for determination of selected pesticides in yogurt samples," *Food Analytical Methods*, vol. 13, no. 10, pp. 1829–1841, 2020.

[8] R. A. Tessema and K. Nagy, "Pesticide use, perceived health risks and management in Ethiopia and in Hungary: a comparative analysis," *International Journal of Environmental Research and Public Health*, vol. 18, no. 19, Article ID 10431, 2021.

[9] IFPRI International Food Policy Research Institute: 2023, https://www.ifpri.org/publication/2020-annual-report.

[10] M. Shamsipur, N. Yazdanfar, and M. Ghambarian, "Combination of solid phase extraction with dispersive liquid–liquid microextraction followed by GC-MS for determination of pesticide residues from water, milk, honey and fruit juice," *Food Chemistry*, vol. 204, pp. 289–297, 2016.

[11] R. Gerber, N. J. Smit, J. H. J. Van Vuren et al., "Bioaccumulation and human health risk assessment of DDT and other organochlorine pesticides in an apex aquatic predator from a premier conservation area," *The Science of the Total Environment*, vol. 550, pp. 522–533, 2016.

[12] M. Mezcua, M. R. Repetti, A. Agüera, C. Ferrer, J. F. García-Reyes, and A. R. Fernández-Alba, "Determination of pesticides in milk based infant formulas by pressurized liquid extraction followed by gas chromatography tandem mass spectrometry," *Analytical and Bioanalytical Chemistry*, vol. 389, no. 6, pp. 1833–1840, 2007.

[13] X. Chen, P. Panuwet, R. E. Hunter et al., "Method for the quantification of current use and persistent pesticides in cow milk, human milk and baby formula using gas chromatography tandem mass spectrometry," *Journal of Chromatography B*, vol. 970, pp. 121–130, 2014.

[14] T. Rejczak and T. Tuzimski, "QuEChERs based extraction with dispersive solid phase extraction clean-up using PSA and ZrO_2 based sorbents for determination of pesticides in bovine milk samples by HPLC-DAD," *Food Chemistry*, vol. 217, pp. 225–233, 2017.

[15] S. Gao, J. You, X. Zheng et al., "Determination of phenylurea and triazine herbicides in milk by microwave assisted ionic liquid microextraction high performance liquid chromatography," *Talanta*, vol. 82, no. 4, pp. 1371–1377, 2010.

[16] X. Wang, X. Meng, Q. Wu, C. Wang, and Z. Wang, "Solid phase extraction of carbamate pesticides with porous organic polymer as adsorbent followed by high performance liquid chromatography–diode array detection," *Journal of Chromatography A*, vol. 1600, pp. 9–16, 2019.

[17] N. Sharma, P. Thakur, and M. G. Chaskar, "Determination of eight endocrine disruptor pesticides in bovine milk at trace levels by dispersive liquid–liquid microextraction followed by GC–MS determination," *Journal of Separation Science*, vol. 44, no. 15, pp. 2982–2995, 2021.

[18] B. Giroud, S. Bruckner, L. Straub, P. Neumann, G. R. Williams, and E. Vulliet, "Trace level determination of two neonicotinoid insecticide residues in honey bee royal jelly using ultra sound assisted salting–out liquid liquid extraction followed by ultra high performance liquid chromatography–tandem mass spectrometry," *Microchemical Journal*, vol. 151, 2019.

[19] D. Chen, Y. Zhao, H. Miao, and Y. Wu, "A novel dispersive micro solid phase extraction using PCX as the sorbent for the determination of melamine and cyromazine in milk and milk powder by UHPLC-HRMS/MS," *Talanta*, vol. 134, pp. 144–152, 2015.

[20] M. Rashidipour, R. Heydari, A. Maleki, E. Mohammadi, and B. Davari, "Salt assisted liquid–liquid extraction coupled with reversed phase dispersive liquid–liquid microextraction for sensitive HPLC determination of paraquat in environmental and food samples," *Journal of Food Measurement and Characterization*, vol. 13, no. 1, pp. 269–276, 2018.

[21] K. Rawat, A. Srivastava, S. Tandon, and G. P. Singh, "Method validation for simultaneous determination of four neonicotinoids in vegetables by liquid chromatography," *Analytical Sciences*, vol. 39, no. 4, pp. 431–439, 2023.

[22] R. Heydari and R. Feyzianpour, "Determination of 2, 4-dichlorophenoxyacetic acid in water and edible seeds samples using saltassisted liquid–liquid extraction coupled with high performance liquid chromatography," *Food Analytical Methods*, vol. 14, no. 3, pp. 561–567, 2021.

[23] S. Seccia, S. Albrizio, P. Fidente, and D. Montesano, "Development and validation of a solid phase extraction method coupled to high performance liquid chromatography with ultraviolet-diode array detection for the determination of sulfonylurea herbicide residues in bovine milk samples," *Journal of Chromatography A*, vol. 1218, no. 9, pp. 1253–1259, 2011.

[24] B. I. Shapiro, G. Gebru, S. Desta et al., "Ethiopia livestock sector analysis: a 15 year livestock sector strategy," Oct. 2017, https://cgspace.cgiar.org/bitstream/handle/10568/92057/LSA_Ethiopia.pdf.

[25] N. Moje, H. Waktole, R. Kassahun et al., "Status of animal health biosecurity measures of dairy farms in urban and peri urban areas of central Ethiopia," *Frontiers in Veterinary Science*, vol. 10, Article ID 1086702, pp. 1–10, 2023.

[26] A. Benedet, "Factors associated with herd bulk milk composition and technological traits in the Italian dairy industry," *Journal of Dairy Science*, vol. 101, no. 2, pp. 934–943, 2018.

[27] S. M. Goulart, M. E. L. R. de Queiroz, A. A. Neves, and J. H. de Queiroz, "Low temperature clean-up method for the determination of pyrethroids in milk using gas chromatography with electron capture detection," *Talanta*, vol. 75, no. 5, pp. 1320–1323, 2008.

[28] A. Campos do Lago, M. H. da Silva Cavalcanti, M. A. Rosa, A. T. Silveira, C. R. Teixeira Tarley, and E. C. Figueiredo, "Magnetic restricted access carbon nanotubes for dispersive solid phase extraction of organophosphates pesticides from

bovine milk samples," *Analytica Chimica Acta*, vol. 1102, pp. 11–23, 2020.

[29] E. M. Shaker and E. E. Elsharkawy, "Organochlorine and organophosphorus pesticide residues in raw buffalo milk from agroindustrial areas in Assiut, Egypt," *Environmental Toxicology and Pharmacology*, vol. 39, no. 1, pp. 433–440, 2015.

[30] J. A. Welsh, H. Braun, N. Brown et al., "Production-related contaminants (pesticides, antibiotics and hormones) in organic and conventionally produced milk samples sold in the USA," *Public Health Nutrition*, vol. 22, no. 16, pp. 2972–2980, 2019.

[31] L. Zhu, K. Huey Ee, L. Zhao, and H. K. Lee, "Analysis of phenoxy herbicides in bovine milk by means of liquid–liquid–liquid microextraction with a hollow fiber membrane," *Journal of Chromatography A*, vol. 963, no. 1–2, pp. 335–343, 2002.

[32] H. R. Nodeh, W. A. Wan Ibrahim, M. M. Sanagi, and H. Y. Aboul-Enein, "Magnetic graphene based cyanopropyltriethoxysilane as an adsorbent for simultaneous determination of polar and non polar organophosphorus pesticides in cow's milk," *RSC Advances*, vol. 6, no. 30, pp. 24853–24864, 2016.

[33] F. D. M. Rodrigues, P. R. R. Mesquita, L. S. de Oliveira et al., "Development of a headspace solid phase microextraction/gas chromatography–mass spectrometry method for determination of organophosphorus pesticide residues in cow milk," *Microchemical Journal*, vol. 98, no. 1, pp. 56–61, 2011.

[34] M. J. González-Rodríguez, F. J. Arrebola Liébanas, A. Garrido Frenich, J. L. Martínez Vidal, and F. J. Sánchez López, "Determination of pesticides and some metabolites in different kinds of milk by solid phase microextraction and low pressure gas chromatography tandem mass spectrometry," *Analytical and Bioanalytical Chemistry*, vol. 382, no. 1, pp. 164–172, 2005.

[35] N. I. Mohd, N. N. M. Zain, M. Raoov, and S. Mohamad, "Determination of carcinogenic herbicides in milk samples using green non ionic silicone surfactant of cloud point extraction and spectrophotometry," *Royal Society Open Science*, vol. 5, no. 4, Article ID 171500, 2018.

[36] Z. Niu, C. Yu, X. He, J. Zhang, and Y. Wen, "Salting out assisted liquid–liquid extraction combined with gas chromatography–mass spectrometry for the determination of pyrethroid insecticides in high salinity and biological samples," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 143, pp. 222–227, 2017.

[37] L. Nováková and H. Vlčková, "A review of current trends and advances in modern bio-analytical methods: chromatography and sample preparation," *Analytica Chimica Acta*, vol. 656, no. 1–2, pp. 8–35, 2009.

[38] A. Tighrine, Y. Amir, P. Alfaro, M. Mamou, and C. Nerín, "Simultaneous extraction and analysis of preservatives and artificial sweeteners in juices by salting out liquid–liquid extraction method prior to ultra high performance liquid chromatography," *Food Chemistry*, vol. 277, pp. 586–594, 2019.

[39] J. M. F. D. Almeida, E. M. F. Silva, L. M. Veríssimo, and N. S. Fernandes, "Salting out assisted liquid–liquid extraction method combined with GC–MS for the determination of topiramate in aqueous solutions: development and application of the methodology," *Separation Science and Technology*, vol. 55, no. 13, pp. 2303–2312, 2019.

[40] S. Akram, B. Sultana, and M. R. Asi, "Salting out assisted liquid–liquid extraction and reverse phase high performance liquid chromatographic monitoring of thiacloprid in fruits and vegetables," *Separation Science and Technology*, vol. 53, pp. 1563–1571, 2017.

[41] J. L. de Oliveira Arias, C. B. Rocha, L. Kupski, S. C. Barbosa, E. G. Primel, and E. G. Primel, "Salting out induced liquid–liquid–liquid microextraction: an environmentally friendly approach to preservative determination in food samples," *Food Analytical Methods*, vol. 14, no. 8, pp. 1625–1636, 2021.

[42] D. Moreno-González and A. M. García-campaña, "Salting out assisted liquid–liquid extraction coupled to ultra high performance liquid chromatography tandem mass spectrometry for the determination of tetracycline residues in infant foods," *Food Chemistry*, vol. 221, pp. 1763–1769, 2017.

[43] A. Lakew, N. Megersa, and B. S. Chandravanshi, "Quantitative extraction of multiclass antibiotic residues in cow milk utilizing salting out assisted liquid–liquid extraction followed by liquid chromatographic determination," *International Journal of Environmental Analytical Chemistry*, pp. 1–19, 2022.

[44] M. A. Farajzadeh, A. Mohebbi, M. R. A. Mogaddam, M. Davaran, M. Norouzi, and M. Norouzi, "Development of salt-induced homogenous liquid–liquid microextraction based on iso–propanol/sodium sulfate system for extraction of some pesticides in fruit juices," *Food Analytical Methods*, vol. 11, no. 9, pp. 2497–2507, 2018.

[45] A. Gure, F. J. Lara, D. Moreno-González, N. Megersa, M. del Olmo-Iruela, and A. M. García-campaña, "Salting-out assisted liquid–liquid extraction combined with capillary HPLC for the determination of sulfonylurea herbicides in environmental water and banana juice samples," *Talanta*, vol. 127, pp. 51–58, 2014.

[46] Y. Wen, J. Li, F. Yang, W. Zhang, W. Li, and C. Liao, "Salting out assisted liquid–liquid extraction with the aid of experimental design for determination of benzimidazole fungicides in high salinity samples by high-performance liquid chromatography," *Talanta*, vol. 106, pp. 119–126, 2013.

[47] Y. Alemayehu, T. Tolcha, and N. Megersa, "Salting out assisted liquid–liquid extraction combined with hplc for quantitative extraction of trace multiclass pesticide residues from environmental waters," *American Journal of Analytical Chemistry*, vol. 08, no. 07, pp. 433–448, 2017.

[48] R. R. Pasupuleti, S. Gurrani, P. Tsai, and V. K. Ponnusamy, "Novel salt assisted liquid–liquid microextraction technique for environmental, food, and biological samples analysis applications: a review," *Current Analytical Chemistry*, vol. 17, pp. 1–11, 2020.

[49] D. Du, G. Dong, Y. Wu, J. Wang, and M. Gao, "Salting-out induced liquid–liquid microextraction based on the system of acetonitrile/magnesium sulfate for trace-level quantitative analysis of fluoroquinolones in water, food and biological matrices by high-performance liquid chromatography with a fluorescence detector," *Analytical Methods*, vol. 6, no. 17, pp. 6973–6980, 2014.

[50] S. Magiera and E. Kwietniowska, "Fast, simple and efficient salting out assisted liquid–liquid extraction of naringenin from fruit juice samples prior to their enantioselective determination by liquid chromatography," *Food Chemistry*, vol. 211, pp. 227–234, 2016.

[51] S. F. Hammad, I. A. Abdallah, A. Bedair, and F. R. Mansour, "Salting out induced liquid–liquid microextraction for alogliptin benzoate determination in human plasma by HPLC/UV," *BMC Chemistry*, vol. 15, pp. 1-2, 2021.

[52] N. H. Sazali, A. Alshishani, B. Saad, K. Y. Chew, and M. M. Chong, "Salting out assisted liquid–liquid extraction coupled with high performance liquid chromatography for

the determination of vitamin D_3 in milk samples," *Royal Society Open Science*, vol. 6, pp. 190952–190958, 2019.

[53] S. Rizzo, M. Russo, M. Labra, L. Campone, and L. Rastrelli, "Determination of chloramphenicol in honey using European commission decision," *Molecules*, vol. 25, pp. 1–13, 2020.

[54] B. T. Negussie, S. Dube, and M. M. Nindi, "Multiclass pesticide residue analysis in fruit and vegetable samples by combining acetone based salting out assisted extraction with dispersive liquid–liquid microextraction," *Journal of Chemistry*, vol. 2021, Article ID 6417093, 12 pages, 2021.

[55] H. Bekele and N. Megersa, "Green analytical method based on salt assisted graphene oxide dispersive solid phase extraction of symmetrical triazine herbicides in environmental water samples for liquid chromatographic determination," *International Journal Environmental Analysis Chemistry*, pp. 1–17, 2023.

[56] European Commission, *Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed*, 2018.

[57] S. Zeiadi, M. R. A. Mogaddam, M. A. Farajzadeh, and J. Khandaghi, "Combination of dispersive solid phase extraction with lighter than water dispersive liquid–liquid microextraction for the extraction of organophosphorous pesticides from milk," *International Journal of Environmental Analytical Chemistry*, vol. 102, no. 17, pp. 5873–5886, 2022.

Appendix: Supplementary material

A Highly Selective Analytical Method Based on Salt Assisted Liquid-Liquid Extraction for Trace Level Enrichment of Multiclass Pesticide Residues in Cow Milk for Quantitative Liquid Chromatographic Analysis

Habtamu Bekele[1], Weldegebrie[l] Yohannes[1], Negussie Megersa[1*]

[1]Department of Chemistry, College of Natural and Computational Sciences, Addis Ababa University,
P. O. Box 1176, Addis Ababa, Ethiopia

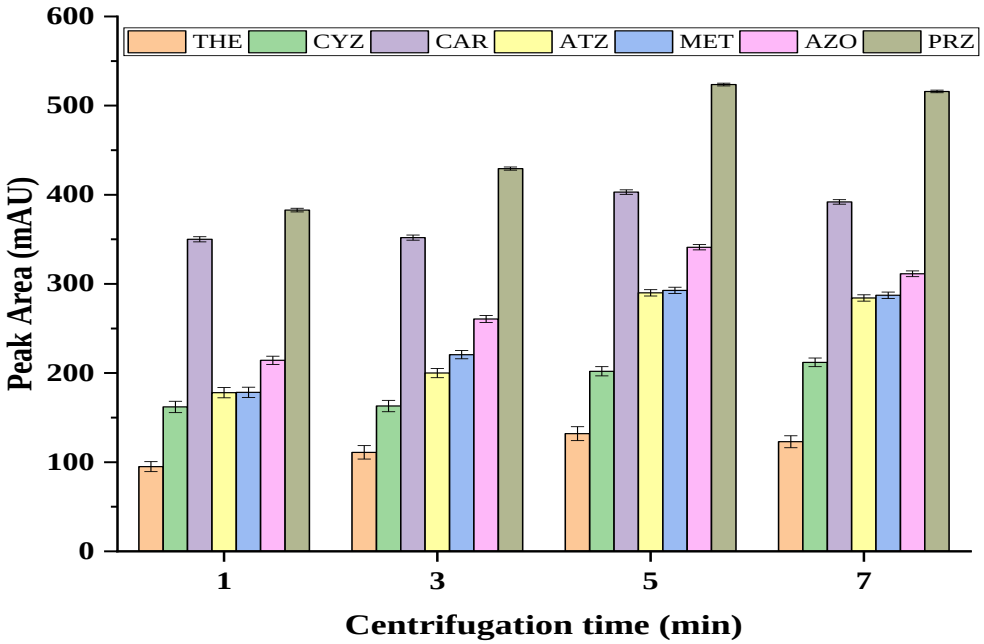
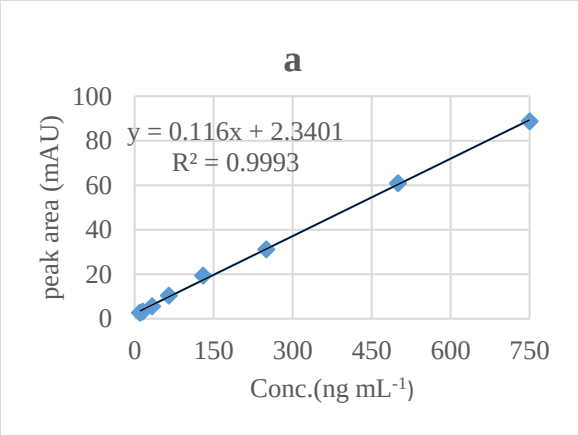
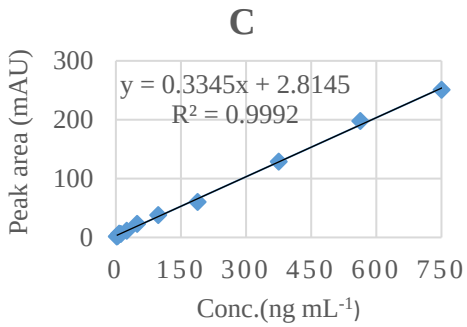
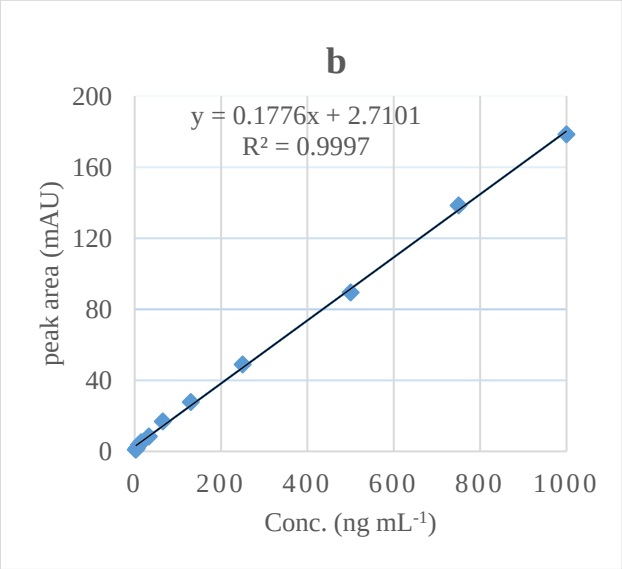
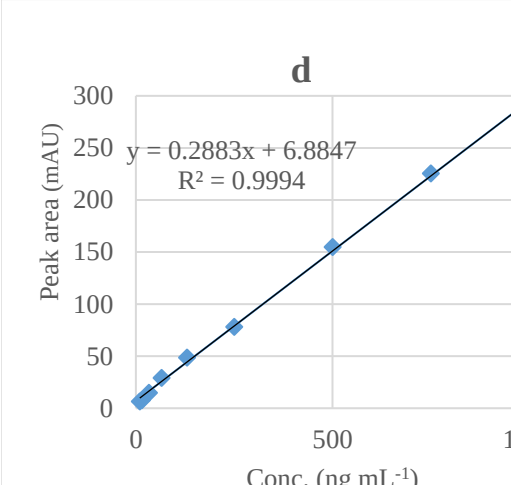


FIGURE S1: Effect of centrifugation time. Extraction conditions: sample size, 5 mL; extraction solvent, acetonitrile; extraction solvent volume; 1000 µL; salt type, $MgSO_4$; amount of $MgSO_4$ added, 40% (m/v); extraction time, 0.5 min; pH of solution, 8.0; centrifugation speed, 4000 rpm, n=6.


a
y = 0.116x + 2.3401
R² = 0.9993
peak area (mAU)
Conc.(ng mL-1)


C
y = 0.3345x + 2.8145
R² = 0.9992
Peak area (mAU)
Conc.(ng mL-1)


b
y = 0.1776x + 2.7101
R² = 0.9997
peak area (mAU)
Conc. (ng mL-1)


d
y = 0.2883x + 6.8847
R² = 0.9994
Peak area (mAU)
Conc. (ng mL-1)

S2

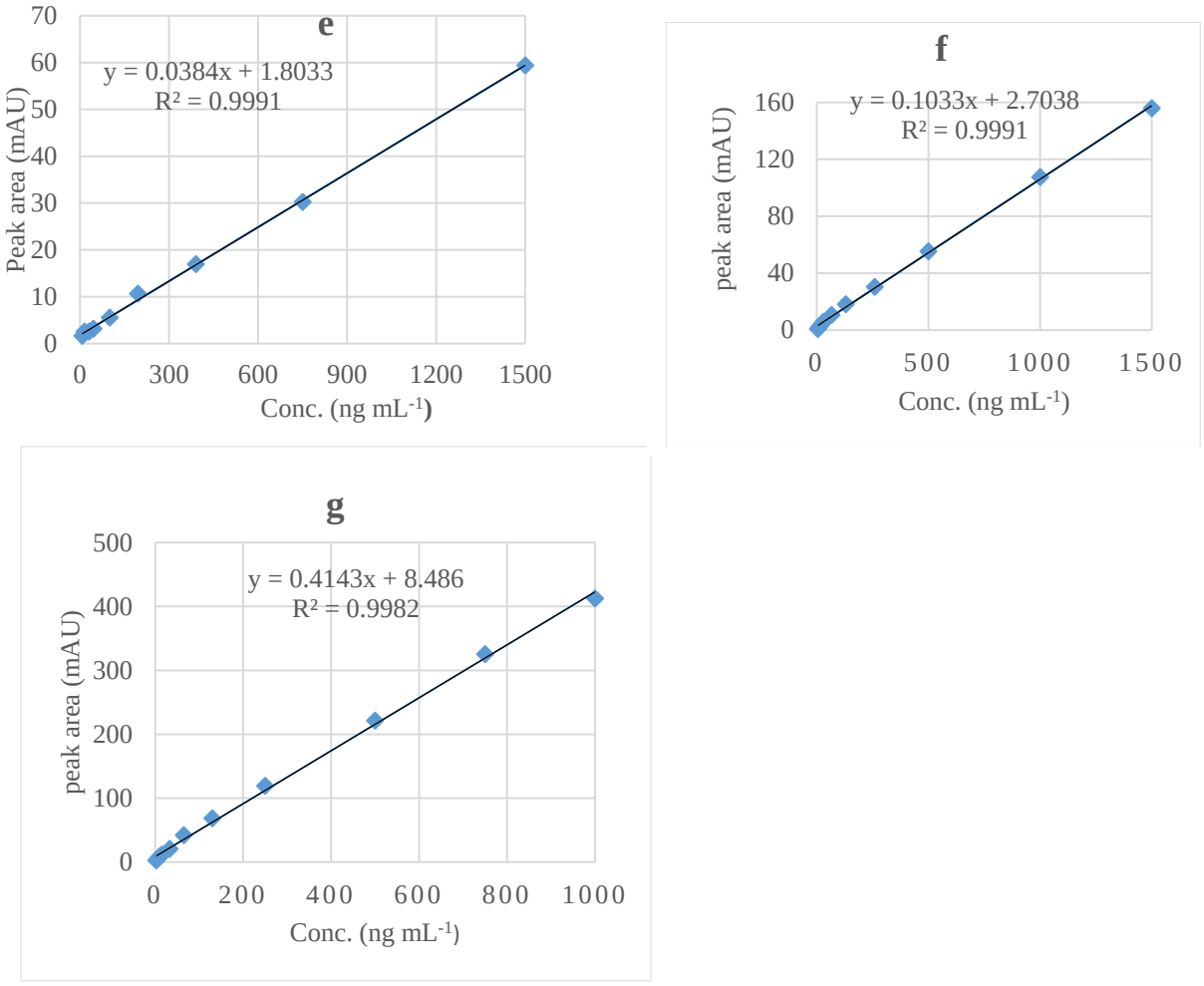


FIGURE S2**:** Calibration curve of a) thiamethoxam, b) cyanazine, c) carbrayl, d) atrazine, e) methidathion, f) azoxystrobin, g) propazine.

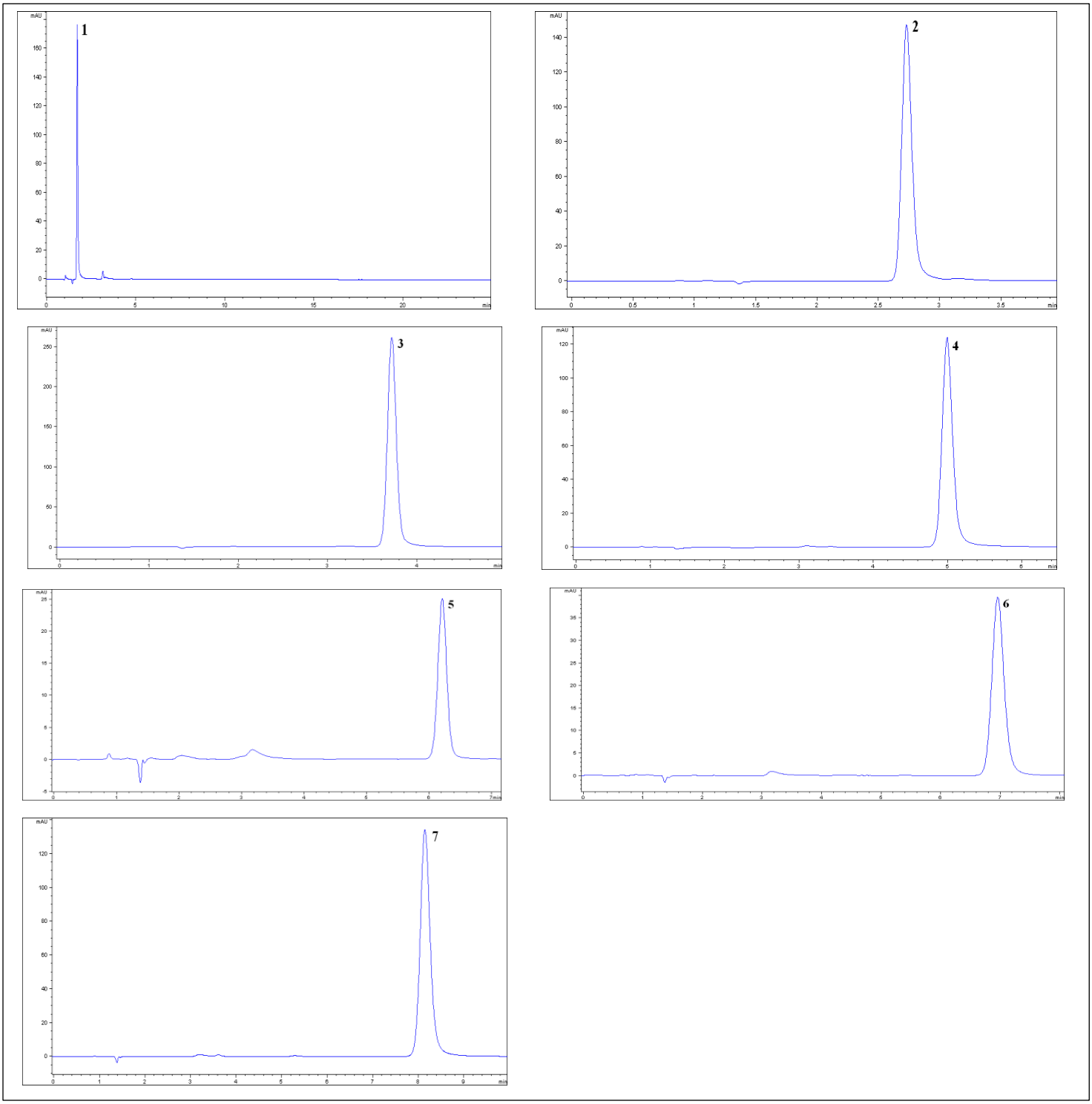


FIGURE S3**:** Chromatograms of individual pesticides: 1, thiamethoxam 2, cyanazine 3, carbrayl 4, atrazine 5, metadiothate 6, azoxystrobin 7, propazine.

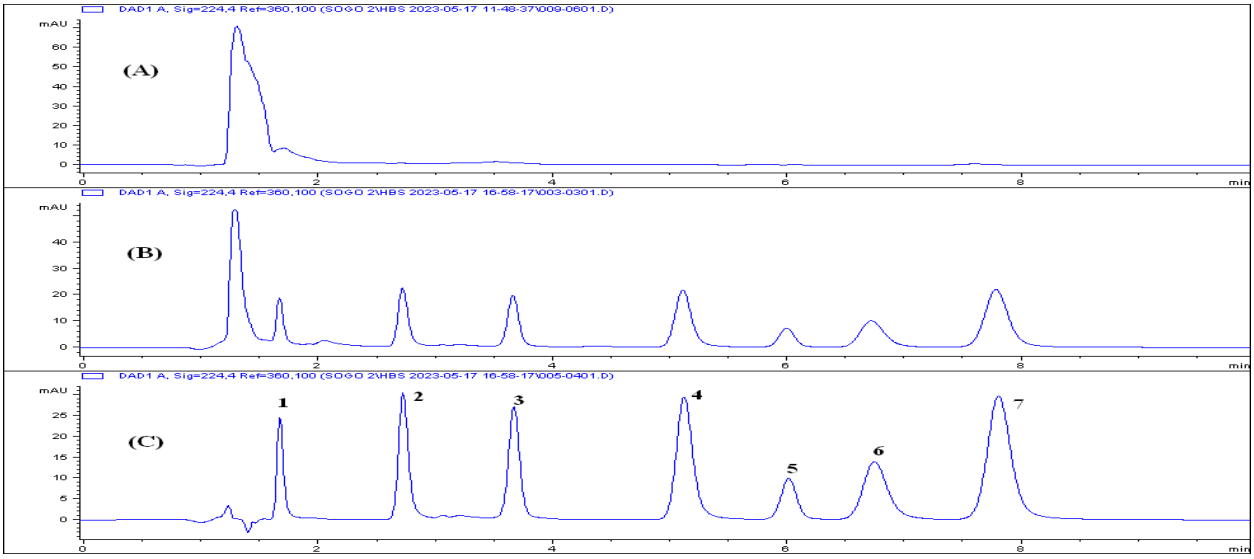


FIGURE S_4**:** Typical chromatograms of blank (A), spiked (B) PMM milk sample, and standard solution (C) at concentration level 2 (100 µg/L for CAR; 130 µg/L for THE, CYZ, ATZ, and PRZ; 260 µg/L for AZO; 390 µg/L for MET). Extraction conditions: sample size, 5 mL; extraction solvent, acetonitrile; extraction solvent volume; 1000 µL; salt type, $MgSO_4$; amount of $MgSO_4$ added, 40% (m/v); extraction time, 0.5 min; pH of solution, 8.0; centrifugation speed, 4000 rpm for 5 min. Peaks identifications: 1, thiamethoxam 2, cyanazine 3, carbrayl 4, atrazine 5, methidathion 6, azoxystrobin 7, propazine.

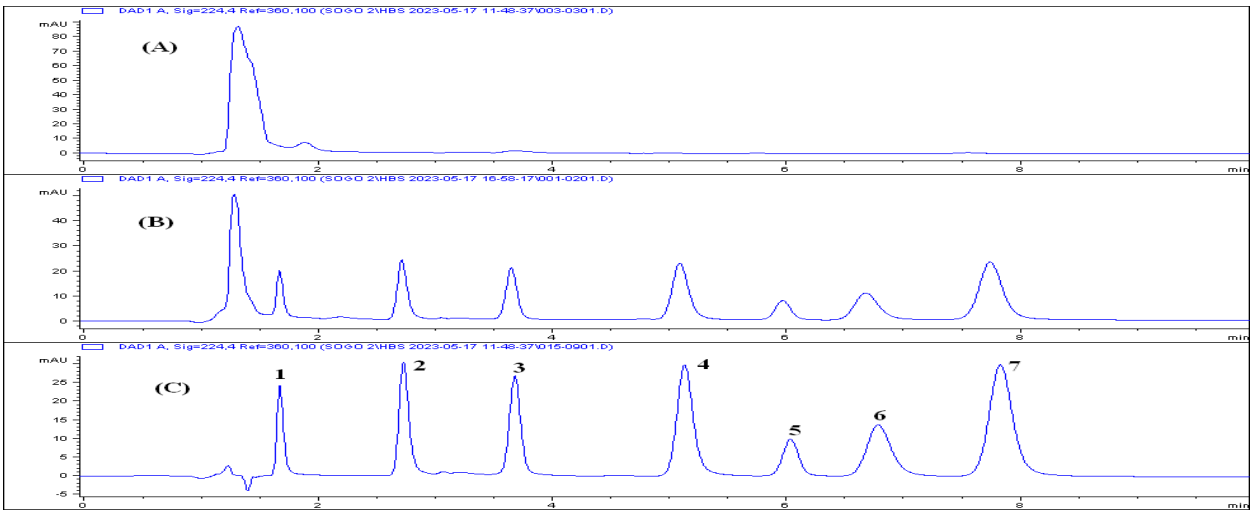


FIGURE S_5**:** of blank (A), spiked (B) RSM milk sample, and standard solution (C) at concentration level 2 (100 µg/L for CAR; 130 µg/L for THE, CYZ, ATZ, and PRZ; 260 µg/L for AZO; 390 µg/L for MET). Extraction conditions: sample size, 5 mL; extraction solvent, acetonitrile; extraction solvent volume; 1000 µL; salt type, $MgSO_4$; amount of $MgSO_4$ added, 40% (m/v); extraction time, 0.5 min; pH of solution, 8.0; centrifugation speed, 4000 rpm for 5 min. Peaks identifications: 1, thiamethoxam 2, cyanazine 3, carbrayl 4, atrazine 5, metadiothate 6, azoxystrobin 7, propazine.

Paper - IV

(Manuscript)

Development of cost effective dispersive liquid–liquid microextraction analytical method for extraction and preconcentration of multiresidue herbicides in environmental water samples prior to chromatographic analysis

Habtamu Bekele, Abi Legesse, Weldegebriel Yohannes, and Negussie Megersa

DEVELOPMENT OF COST EFFECTIVE DISPERSIVE LIQUID–LIQUID MICROEXTRACTION ANALYTICAL METHOD FOR EXTRACTION AND PRECONCENTRATION OF MULTIRESIDUE HERBICIDES IN ENVIRONMENTAL WATER SAMPLES PRIOR TO CHROMATOGRAPHIC ANALYSIS

Habtamu Bekele, Abi Legesse, Weldegebrie Yohannes, Negussie Megersa*

Department of Chemistry, College of Natural and Computational Sciences, Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia

Corresponding Author:

Prof. Negussie Megersa

Email: negussie.megersa@gmail.com

ORCID: https://orcid.org/0000-0003-1255-7554

P. O. Box (Personal): 34706, Addis Ababa, Ethiopia

Telephone (mobile): +251-911-68-42-60

Fax: +251-111-23-94-70

Co-authors:

1. Habtamu Bekele
   Email: bekelehabtamu16@gmail.com
2. Abi Legesse
   Email: abi.legesse@yahoo.com
3. Weldegebriel Yohannes
   Email: wyohannes2001@gmail.com

ABSTRACT

High density dispersive liquid–liquid microextraction method coupled high-performance liquid chromatography with diode array detector (HD-DLLME-HPLC-DAD) was developed for extraction and determination of six commonly used sulfonylurea herbicides in matrices of environmental waters. For simultaneous extraction of target herbicides, the optimum experimental parameters that influenced extraction efficiency were investigated. Under optimized conditions, low limits of detection (LODs) in the range of 0.8–1.5 ng mL^{-1}, and limits of quantification (LOQ) in the range of 1.9–5.1 ng mL^{-1} were obtained. The precisions in terms of relative standard deviations (% RSDs) of intra-day (n = 6) and inter-day (n = 9) were found to be 3.01 to 8.36 and 2.92 to 9.78, respectively. Furthermore, applicability of developed method was investigated by analyzing tap, lake, river and underground water samples spiked and satisfactory recoveries were obtained in the range of 84.3–101.7% with RSDs < 9.8% (n=6) and the target analytes were not detected in real samples. The proposed method offered a number of attractive features of fast analysis time, simplicity, sensitivity, and selectivity. Therefore, the trace level enrichment and assessment of sulfonylurea herbicides residues from environmental water matrices using HPLC-DAD could be utilized as reliable alternative in regular laboratory analysis.

KEYWORDS: Environmental water, High density dispersive liquid–liquid microextraction, HPLC-DAD, Miniaturization analytical technique, Sulfonylurea herbicides, Trace level enrichment.

INTRODUCTION

Sulfonylurea herbicides, a large family of herbicides used extensively in agriculture, have gained quite significant attention all around the world due to their broad-spectrum and high herbicidal activity at low dosage application rates (10-40 g ha^{-1}), good crop selectivity, and low mammalian toxicity as a result of their low application rates [1, 2]. Depending on the pH, sulfonylurea herbicides (SUHs) which are susceptible to contraction of the sulfonylurea linkage degrade in water 10 to1000 times faster than the others [3]. In addition, although the application rates are low, due to relatively high solubility in water and moderate to high mobility, these herbicides may result in leaching into deeper soil and potentially entering surface waters [4, 5]. Despite the fact that sulfonylurea herbicides rapidly decompose in water and soil due to their thermal and chemical instabilities, they still exist in some situations at a trace level [6] and are being detected in surface and ground waters [7, 8]. Therefore, the presence of significant levels of pesticide residues in environmental matrices has emerged to pose serious environmental and human health problems [9, 10].

The need to develop a reliable analytical method for identifying and determining the presence of SUH residues in environmental and food samples derives from the possibility that SUH residues in the environment could be harmful to animals and plants. Several analytical techniques developed for determining their residues including gas chromatography coupled with mass spectrometry (GC-MS) [11], high performance liquid chromatography equipped with multi wavelength or ultraviolet detector (HPLC-DAD/UV) [1, 12–15], HPLC coupled to tandem mass spectrometry (HPLC-MS/MS)[1, 3, 7, 16], and HPLC coupled to mass spectrometry (HPLC-MS) [17], etc have successfully been applied. Since most of SUHs are unstable at high temperature, a time consuming procedure of sample derivatization or hydroxylation was required prior to analysis with gas chromatography, which limited the application of these detection methods in determination of SUHs. HPLC-UV/DAD and HPLC-MS can be used directly for analysis without derivatization, despite the volatility or heat stability of these analytes. HPLC-UV/DAD is a fast, simple, easy to use and widely available technique, which is usually chosen in the determination of SUHs.

Since the occurrence of SUHs in the environmental samples is at a trace level, the sample pretreatment is needed to extract and enrich them before analysis. Liquid-liquid extraction (LLE) [18, 19] and solid phase extraction (SPE) [7, 8, 15] were the most commonly used techniques in sample pretreatments. LLE can offer high reproducibility and high sample capacity, but suffer from the disadvantages of

requiring long processing time and utilize large volume of sample and also use large quantity toxic organic solvents. SPE can overcome some disadvantages of LLE such as high consumption of both sample and organic solvent, but it also requires longer experimental time because of column conditioning, washing, loading and elution. On the other hand, sorbent ready to use in SPE is relatively expensive, the problem associated to the sample carry over effect which causes increased cost of sample handling and identifying appropriate sorbents during multiclass trace level analysis [20, 21].

To prevent crop loss and improve the quality of the final product, pesticides are purposefully applied to crops. The produce still contains trace pesticide residues, which eventually threaten human health. To deal with the increasing utilization of pesticides, there were quite a number of research conducted on the effects of these chemicals on human health and the environment, as the result legislation are continually being developed, revised and issued. Environmental issues and the high costs of pesticide residue monitoring programs motivate analytical chemists to develop new and improve existing methods. The sample size, cost, and time were reduced by the use of miniaturization methods, which are also safe for the entire environment.

Very recently large group of researches [22, 23] have been focused and committed on the development of efficient, inexpensive, automated, and miniaturized extraction techniques that might substantially reduce consumption of toxic organic solvents. To this end, single drop microextraction (SDME) [24], hollow-fiber liquid phase microextraction (HF-LPME) [12, 25] and solid phase microextraction (SPME) [26, 27] techniques significantly minimized and working towards avoiding the use of organic solvents in sample preparation procedures. However, SPME is expensive, fiber is fragile, has a short lifetime and takes a long time to condition the sorbent [27]. Despite its ease of use and effectiveness, SDME is merely used for laboratory research due to its drop instability and the main drawbacks of HF-LPME are poor reproducibility and lengthy equilibration times [21].

To overcome these limitations, Assadi and coworkers developed dispersive liquid-liquid microextraction (DLLME) technique [28]. It is a modified version of solvent extraction which has the ability of enhancing enrichment intensely, and contains very small amount of toxic solvent used and is the technique in which acceptor to donor phase ratio is greatly reduced compared to other methods used for similar purposes [23, 29, 30]. The extraction and dispersive solvents are promptly injected into the

aqueous sample to create a cloudy solution, which is an essential component of the trace enrichment principle in DLLME. Due to high surface interactions between the droplets of the extraction solvent and aqueous sample solution, extraction equilibrium is quickly attained [22, 31]. Furthermore, as a microextraction technique, DLLME provides features such as ease of use, fast, cost-effectiveness, high recovery, and the use of inexpensive, widely accessible laboratory supplies [32]. The technique has commonly been used to extract trace levels of pesticides ever since it was first introduced, mostly from water samples [29, 33–35], foods [36–38], and especially from juices and vegetables [39, 40]. However, there are very few literature reports on application of the technique for determination of SUHs in waters and none on the use of high density solvent based dispersive liquid-liquid microextraction (HD-DLLME), for simultaneous extraction of these six target SUH residues (metsulfuron-methyl, chlorosulfuron, niclosulfuron, prosulfuron, flazasulfuron and chlorimuron-ethyl) from environmental water samples of different sources. Hence, the objective of this study was to develop, optimize and assess the accuracy and applicability of the method by employing the simplicity and high efficiency of HD-DLLME for trace extraction and enrichment of selected six sulfonylurea pesticide residues in the matrices of environmental waters (tap, underground, lake, and river) prior to chromatographic analysis.

EXPERIMENTAL

Chemicals and reagents

Analytical standards of metsulfuron-methyl (MSM), chlorosulfuron (CS), niclosulfuron (NS), and prosulfuron (PS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Flazasulfuron (FS) and chlorimuron-ethyl (CME) were purchased from Chem Service Inc. (West Chester, USA). All the standards were of the highest purity, i.e., > 97 %. Other chemicals used in the study were also of analytical grade reagents while the solvents utilized including acetonitrile (ACN) and acetone, acquired from Sigma Aldrich (Steinheim, Germany), methanol (MeOH) received from Carlo Erba (Rodano, Italy) and isopropanol (IPA) and chloroform are the product of Sigma Aldrich (Seelze, Germany) were of HPLC grade reagents. Dichloroethane was the product of avocado research chemicals Ltd. (Cheshire, UK). Common chemicals such as NaCl was obtained from Sigma-Aldrich (Steinheim, Germany) and sodium hydroxide (NaOH) was the product of Merck Chemicals (Darmstadt, Germ any). Hydrochloric acid (HCl) was purchased from Sigma Aldrich (St. Louis, MO, USA). Ultrapure water was obtained by

purifying with double distiller, A 8000 Aquatron water Still (Bibby Scientific, Staffordshire, UK) and deionizer (EASY Pure LF, Dubuque).

Instruments and equipment

Chromatographic analyses were carried out using Agilent 1200 series HPLC system (Agilent Technologies, Waldbronn, Germany) outfitted with a quaternary pump, vacuum degasser, standard and preparative autosampler, thermostat column compartment, autosampler thermostat, and a diode array multiple wavelength detector. LC Chemstation software (B.02, 01-SR1) were used for sample processing, and data acquisition. Chromatographic separation was performed using a ZORBAX ODS-C_{18} (150 x 3 mm, i.d., 3.5 µm particle size) analytical column from Agilent Technologies. The sample solution pH was measured using Adwa pH meter, model 1020, made by Adwa Hungary Kft, in Szeged, Hungary. For sample preparation, XW-80A vortex, Jing Industrial Co., Ltd. (Shanghai, China), a centrifuge, Model 80-2, Jiangsu Zhenji instruments Co., Ltd. (Jiangsu, China), a 15 mL centrifuge tube, Corning integrated (Corning, NY, Mexico), and ultrasonic heater, Dacon®, were utilized.

Chromatographic conditions

Chromatographic separations were achieved using isocratic delivery mode with binary mobile phase, ~~of~~ both containing 0.01% acetic acid, i.e., solvent A (ultrapure water) and solvent B (Acetonitrile) in a 1:1 volume ratio. Prior to the sample injection, the HPLC column was equilibrated with the mobile phase for 15 min. Analysis was performed with the flow rate of 1 mL/min, column temperature at 30°C, injection volume of 15 µL and UV detection was performed at 230 nm with bandwidth of 4 in reference to wavelength 360 nm having bandwidth 100 for all the target analytes. Peak area was used as instrumental response and for comparison of the responses. Under these chromatographic conditions, baseline separation was maintained for all the target analytes in 8 min run time.

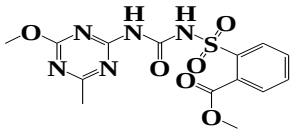
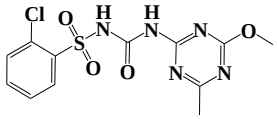
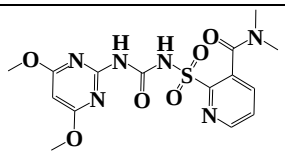
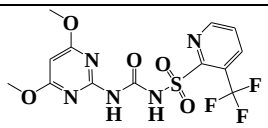
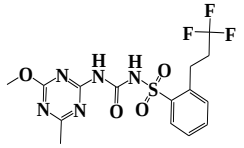
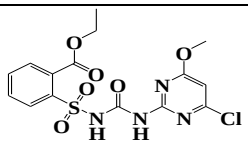
.

Standard solution preparation

The stock standard solution of each target analyte, with the concentration of 500 µg/mL, was prepared by weighing 12.5 mg and dissolving with acetonitrile (ACN) in 25 mL volumetric flask. Intermediate standard solutions of 20 µg/mL was obtained by diluting the stock solution with ACN. Other working solutions of lower concentrations were prepared from the intermediate solution in the same solvent. All standard solutions were stored in the refrigerator at 4°C, when not in use. The chemical structures,

common names, abbreviations and the octanol water partition coefficient (log K_{ow}) at pH 7 and 20°C, and other relevant physicochemical properties of the target pesticides are shown in Table 1.

Table 1. Physicochemical properties of target analytes of the sulfonylurea herbicides [5].

Name	Structure	Solubility, mg/L (25°C, pH 7)	pK_a	log K_{ow} (pH 7)
Metsulfuron-methyl		2790	3.3	1.74
Chlorosulfuron		31800	3.6	-0.99
Niclosulfuron		12000	4.3	-1.8
Flazasulfuron		2100	4.37	-0.06
Prosulfuron		4000	3.76	-0.21
Chlorimuron-ethyl		1200	4.2	0.36

Environmental water samples

Various types of environmental water samples were collected from Bishoftu town which is found in Oromia regional state, Ethiopia with geographical location 8°44’40’’N latitude and 38°59’9’’E longitude at an altitude of 1925 meters above sea level. Samples of Hora lake water, tap water, and underground water were collected from this area. River water was also collected from an area located 10 km away from Bishoftu town, Mojo River, where more than 15 floriculture industries established around and effluents of floriculture industries are directly discharged to the river. Before analysis, each water sample

was filtered through 0.45 μm micropore membrane filter and then stored in a polyvinyl chloride bottles, in a refrigerator, for a maximum of 24 h at 4°C without any further sample pretreatment.

HD-DLLME extraction procedure

Water samples were filtered through Whatman filter paper and 5 mL portions of each type of water sample, adjusted to pH 2, was measured and transferred into a 15 mL falcon tube with conical bottom. Afterwards, it was fortified with appropriate amount of mixture of each target analytes and left to stand for about 15 min for equilibration. Then, before using the sample for DLLME, aqueous solution of 15% (w/v) NH_4AC (ammonium acetate) was added and vortex agitated for 1.5 min to dissolve in water sample. Subsequently, the organic phase consisting of a mixture of 800 μL MeOH and 175 μL dichloroethane was injected into the sample solution with 2 mL syringe. The mixture was vortexed for 0.5 min at the highest speed followed by centrifugation of the content at 4,000 rpm for 5 min to enhance sedimentation of the fine organic droplets. The organic phase of the sediment was carefully removed using 1 mL syringe, transferred to a 1.5 mL glass vial, and allowed to air dry at room temperature. The residue was ultimately redissolved in 300 μL of solvent containing 0.01% acetic acid and 0.01% acetic acid acetonitrile (1:1, v/v) mixture, followed by vortexing for 1 min. The resulting solution was subsequently filtered with a 0.22-μm nylon filter and transferred to 1.5 mL vial and finally placed on the autosampler from which, 15 μL solution was injected into the HPLC and determination of the target analytes were achieved.

RESULTS AND DISCUSSIONS

Parameter Optimization

The most important variables that affect extraction efficiency, such as sample pH, ionic strength, and vortex (extraction time), as well as the type and volume of extraction and dispersive solvents, were investigated to determine the optimal conditions using the univariate approach. By adding 200 ng/mL working standard solutions to 5 mL of ultrapure water, each experiment was carried out at least in triplicate. The average peak areas of the replicate analyses were taken into account to assess the impact of experimental circumstances on the extraction efficiency in any given procedure. Preliminary experimental results and review of related literatures were used to determine quantitative value ranges of parameters optimization.

Influence of extraction solvent type

To achieve a successful extraction in the DLLME process, the choice of the appropriate extraction solvent is of utmost significance. When injected into the aqueous solution, the chosen solvent should have a density greater than water, produce a stable two phase system in the presence of a dispersive solvent, and demonstrate strong extraction capability for the target analytes [37]. In the present study, four organic solvents such as chloroform, dichloromethane, dichloroethane and chlorobenzene were tested by injecting a mixture of equal volume of each of these alternative extraction solvents and the same amount of methanol as dispersive solvent. It was observed that for all organic extraction solvents a phase separation were achieved. However the,-highest peak area for most target analytes were obtained with dichloroethane as shown in Figure 1. Therefore, dichloroethane was chosen as extraction solvent for further studies.

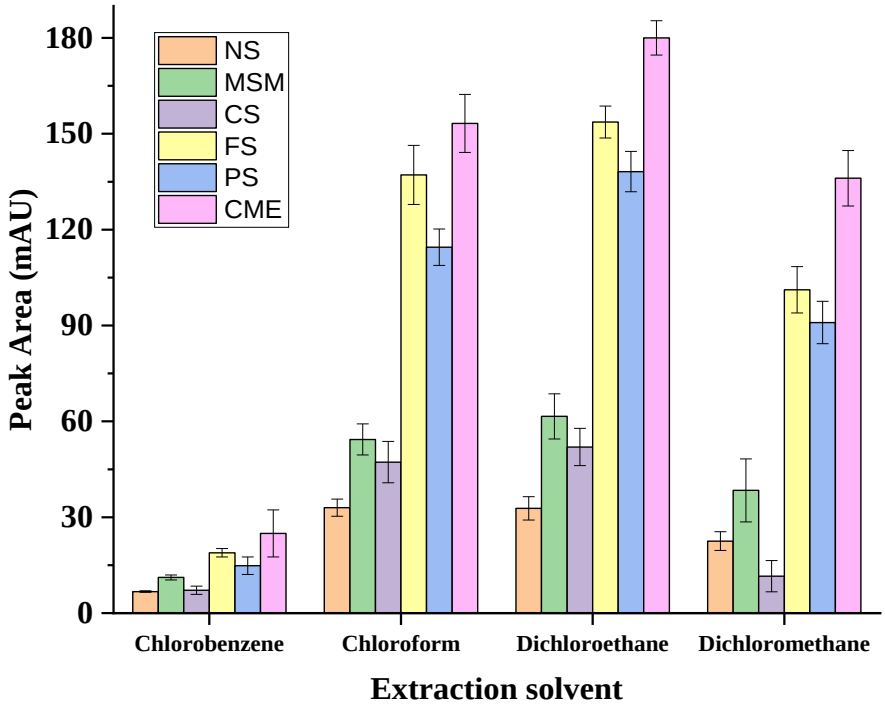


Figure 1. Effect of extraction solvent type. Extraction conditions: sample size, 5 mL; dispersive solvent, methanol; dispersive solvent volume, 800 µL ; salt type and amount added, NH_4AC (15% m/v); pH of solution, 2.0; vortex agitation time, 0.5 min; volume of extraction solvent, 800 µL; centrifugation speed 4000 rpm for 5 min; n=3.

Effect of extraction solvent volume

The effect of extraction solvent volume on the extraction efficiency was evaluated by varying the volume in the range of 50–200 µL, while other experimental parameters were kept constant. It was observed that the extraction efficiency of DLLME procedure was meaningfully affected by the volume of extracting solvent. As can be seen in Figure 2, the peak area of all the target analytes increased with the volume of dichloroethane up to 175 µL, then decreased for higher value, i.e., 200 µL which may be due to dilution effect of the sediment phase of extraction solvent [41]. Thus, 175 µL was selected as optimum volume of extraction solvent.

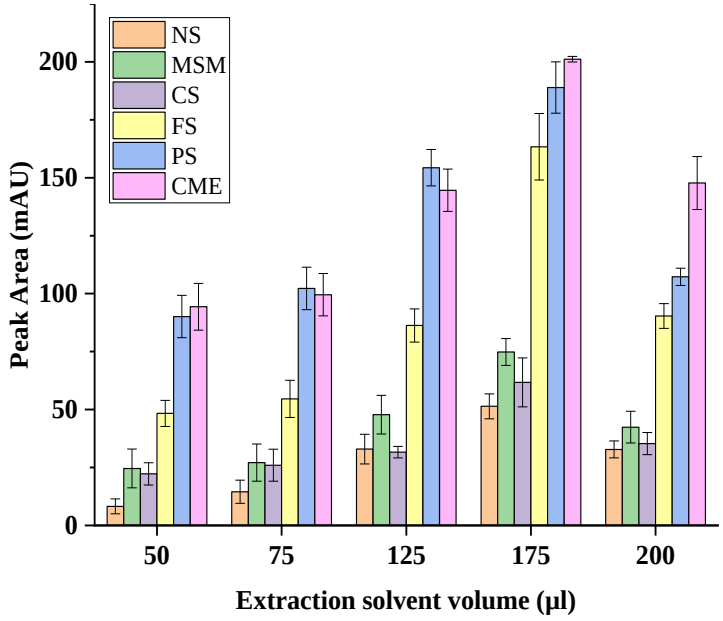


Figure 2. Effect of extraction solvent volume. Extraction conditions: sample size, 5 mL; Extraction solvent, dichloroethane; dispersive solvent, methanol; dispersive solvent volume, 800 µL; salt type and amount added, NH_4AC (15% m/v); pH of solution, 2.0; vortex agitation time, 0.5 min; centrifugation speed, 4000 rpm for 5 min; n = 3.

Selection of dispersive solvent type

The type of dispersive solvent was studied to get the optimum extraction efficiency. Dispersive solvents often need to be miscible with the extraction solvent as well as the sample solution [35, 37]. In the current study, four solvents including methanol (MeOH), isopropanol (IPA), acetone and acetonitrile (ACN) were evaluated. The highest peak areas for all the SUHs were obtained with MeOH followed by

IPA (Figure 3). This phenomenon could be due to the high polarity of these solvents caused by their hydroxyl group and the hydrogen bond formed between these solvents with dichloroethane and water which as a result increases their miscibility in extraction solvent and the sample solution [42]. The same solvent was also reported as dispersive for extraction of SUs from wine, water and soil samples [14, 43]. Therefore, MeOH was thus chosen as dispersive solvent in this studies.

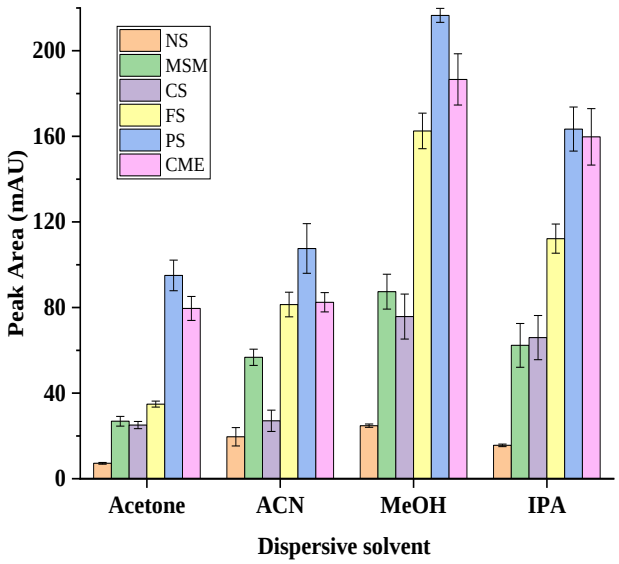


Figure 3. Effect of dispersive solvent type. Extraction conditions: Sample size, 5 mL; extraction solvent, dichloroethane; extraction solvent volume, 175 µL; dispersive solvent volume, 800 µL; salt type and amount added, NH_4AC (15% m/v); pH of solution, 2.0; vortex agitation time, 0.5 min; centrifugation speed, 4000 rpm for 5 min; n = 3.

Effect of dispersive solvent volume

Dispersive solvent volume is another crucial factor that affects the solubility of the extraction solvent in the aqueous sample and thus, can affect the extraction efficiency of the target analytes [9]. The volume of methanol tested was varied in the range of 600–1200 µL with 200 µL interval. As can be seen in Figure 4, the extraction efficiency increases with the volume of methanol up to 800 µL and then decreases at higher volumes. The most probable reason could be associated to the solvent volume, i.e., at low dispersive solvent volume, the organic extractant sediment phase might not be formed properly, giving a low peak area. On the other hand, the use of a higher volume of methanol could enhance the solubility of the analytes into the aqueous phase due to the increase of partitioning of the dispersive

solvent in the aqueous sample, leading to lower extraction efficiencies [31]. Based on the experimental results, 800 µL methanol was chosen as optimum.

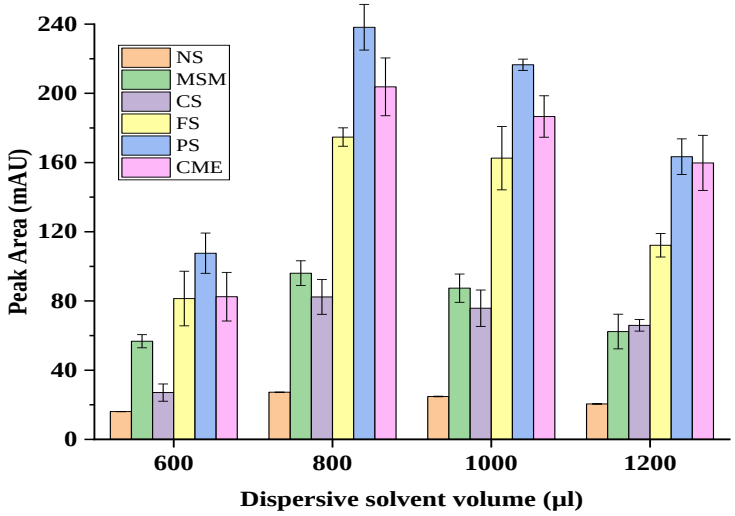


Figure 4. Effect of dispersive solvent volume. Extraction conditions: Sample size, 5 mL; extraction solvent, dichloroethane; extraction solvent volume, 175 µL; dispersive solvent, methanol; salt type and amount added, NH_4AC (15% m/v); pH of solution, 2.0; vortex agitation time, 0.5 min; centrifugation speed, 4000 rpm for 5 min; n = 3.

Sample solution pH

The pH of the aqueous sample solution is a crucial aspect to take into account for acidic or basic analytes. The SUHs used during this investigation are slightly acidic compounds, with pKa values ranging from 3.6 to 5.2 [36]. Therefore, to convert them into their neutral forms and enhance the affinity of the analytes for the extraction solvent, pH of the aqueous solution must be lower than their pK_a values [1]. Hence, the impact of sample pH was considered and optimized over the range 1–5, keeping the other experimental parameters constant. In a more acidic solution, however, lower peak areas were observed probably due to the fact that a very acidic pH could accelerate hydrolysis of the pesticide compounds [2]. The results indicated that the peak area increases when pH raises from 1 to 2 and then decreases by increasing pH from 2 to 5 (Figure 5). At higher pH, the target analytes might not be completely transformed to their neutral forms and thus complete transfer of the analytes from the sample solution to the organic phase may not be achieved. As a consequence, in the next experiments the pH of sample solutions were set at 2.0. Similar findings were also reported by other workers [14, 44].

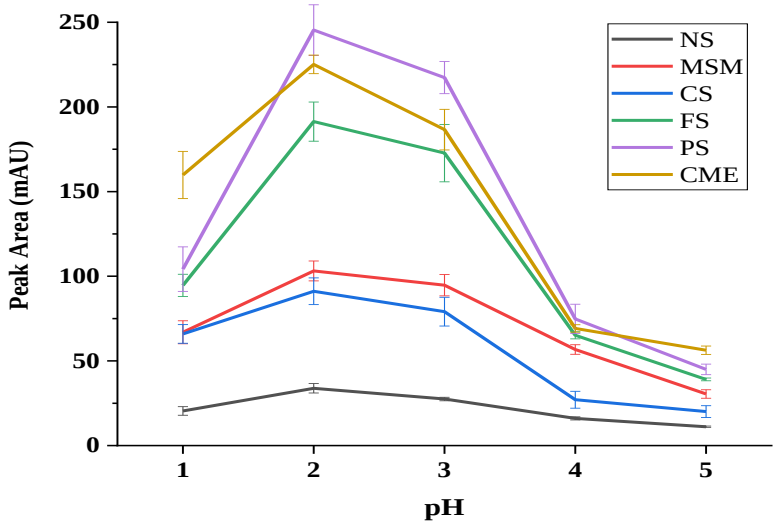


Figure 5. Effect of pH value. Extraction conditions: Sample size, 5 mL; extraction solvent, dichloroethane; extraction solvent volume, 175 µL; dispersive solvent, methanol; dispersive solvent volume, 800 µL; salt type and amount added, NH_4AC (15% m/v); vortex agitation time, 0.5 min; centrifugation speed, 4000 rpm for 5 min; n = 3.

Effects of the salt type

Solubility of both the analytes as well as the extraction solvent in the aqueous phase could be decreased by salt addition and this in turn enhances the analytes transfer into the organic phase [34]. As different salts have the capacity to cause different degrees of phase separation [45], in this study, the effect was evaluated by addition of the different salts such as NaCl, $(Na)_2SO_4$, $MgSO_4$, and ammonium acetate (NH_4AC), using 15% (m/v) of each salt, as potential salting out agent. It was observed that except $(Na)_2SO_4$ other salts induce very clear phase separation, but as it can be seen in Figure 6, the highest instrumental response for all of the analytes were obtained when NH_4AC was used as the salting out agent.

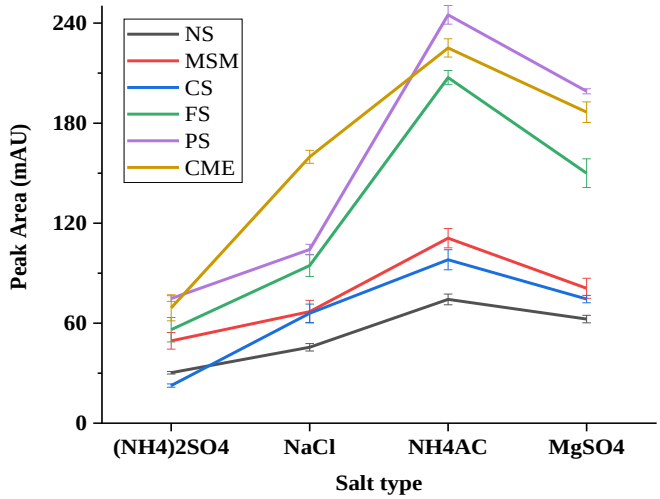


Figure *6.* Effect of salt type. Extraction conditions: sample size, 5 mL; extraction solvent, dichloroethane; extraction solvent volume, 175 µL; dispersive solvent, methanol; dispersive solvent volume, 800 µL; pH of solution, 2.0; amount of salt added, 15% (m/v); vortex agitation time, 0.5 min; centrifugation speed, 4000 rpm for 5 min; n = 3.

Effect of salt concentration

Appropriate quantity of inorganic salts can improve extraction efficiency by increasing the polarity of the water phase and reducing solubility of the target analytes in the aqueous phase [34]. Varying salt concentrations may cause the degrees of phase separation to vary [32]. A salting out study was carried out by adding different amounts of NH_4AC in the range of 0.5–1.5 g (or 10–25%, m/v), in the aqueous sample solution. It was shown that in Figure 7, the peak area of the target analytes were increased as the concentration of the salt increases from 0.5 – 0.75 g. However, at higher concentrations, the peaks were observed to decrease slightly for all the target analytes and, thus 15 % m/v (0.75 g) was chosen to be the optimum for the subsequent experiments. Excessive inorganic salts will increase the viscosity of the solution, resulting in a lower diffusion coefficient and further reducing the extraction efficiency of the target analytes.

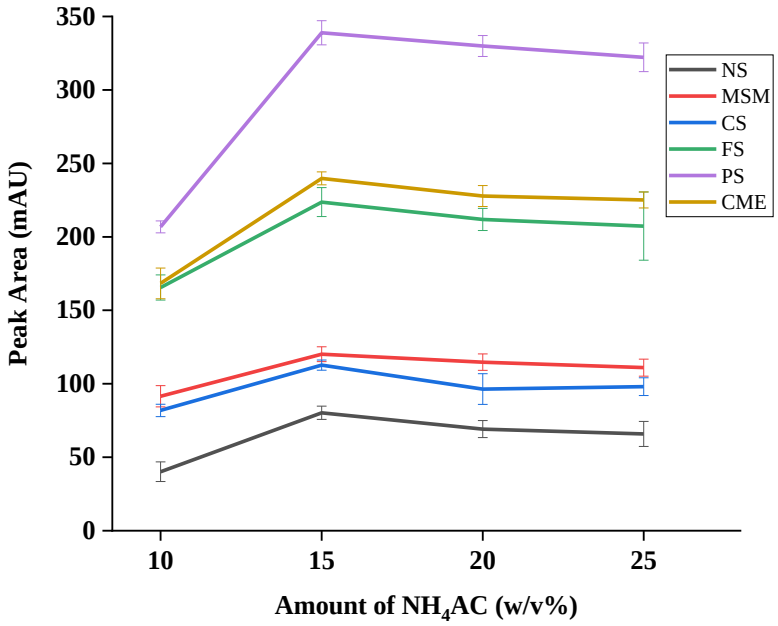


Figure 7. Effect of the salt amount. Extraction conditions: sample size, 5 mL; extraction solvent, dichloroethane; extraction solvent volume, 175 µL; dispersive solvent, methanol; dispersive solvent volume, 800 µL; pH of solution, 2.0; salt type, NH_4AC; vortex agitation time, 0.5 min; centrifugation speed 4000 rpm for 5 min; n = 3.

The influence of vortex time on the extraction

In order to speed up the production of turbid dichloroethane solution, vortex agitation is primarily used. This study investigated the extraction efficiency as a function of vortex time, which was increased from 0.25 to 2 min with intervals of 0.5 min while maintaining a constant vortex rotation speed. The highest extraction efficiency was attained for most analytes after 0.5 min of vortex duration. The high contact surface between the extractant and the aqueous sample may have contributed for this rapid equilibriums achievement [31]. However, as the vortex time was extended, there was little to no noticeable difference as results of these experiment, as were also represented in Figure 8. Thus, a vortex time of 0.5 min was found to be the optimum extraction time.

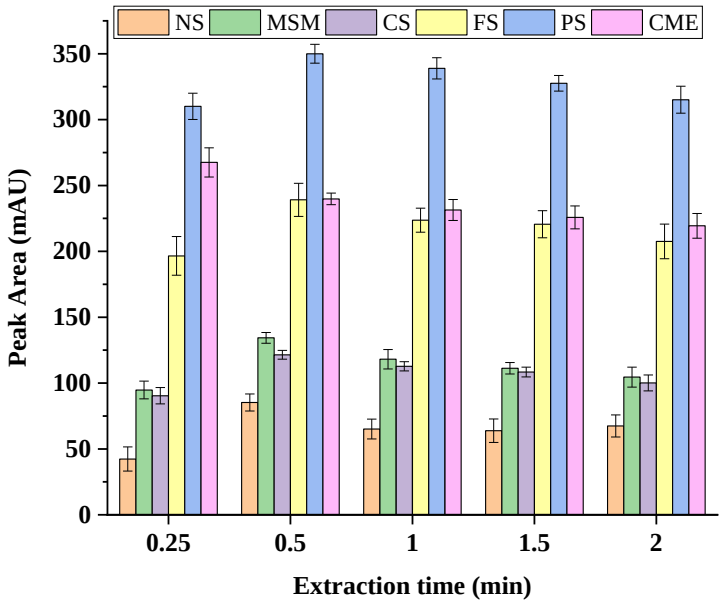


Figure 8. Effect of extraction time. Extraction conditions: sample size, 5 mL; extraction solvent, dichloroethane; extraction solvent volume, 175 µL; dispersive solvent, methanol; dispersive solvent volume, 800 µL; pH of solution, 2.0; salt type and amount, NH_4AC (15% m/v); centrifugation speed 4000 rpm for 5 min; n = 3.

Analytical performance of the proposed method

Before applying the whole procedure to real samples, all the crucial analytical parameters were determined for each pesticide studied such as the linear range (LR), the correlation coefficient (r^2), the limits of detection (LODs), the limits of quantification (LOQs), the inter and intra-day precisions. The entire procedure was tested for real sample solutions, i.e. lake, river, underground and tap waters. But, precision study of the river water was taken as the model representative of all real water samples after spiking with two concentration levels (Level 1 and Level 2; as shown in Table 2) of the working standard solution. The solutions were separately processed according to the DLLME procedure and HPLC-DAD analysis stated in the experimental section.

Calibration curves and precision study

Under optimal conditions, analytical figures of merit were used to validate the proposed analytical methodology. The methods linearity was validated via linear calibration obtained by fortification of each real sample with standard solutions, and the corresponding curves were generated by plotting analyte

extract peak areas against the standard concentration between 2.5 and 1000 (ng/mL). All of the target analytes taken into account in this study has shown good linearity, with correlation coefficients > 0.997 (Table 2).

The repeatability (intra-day precision) and reproducibility (inter-day precision) of the proposed method precision were also assessed. River water was spiked with a mixture of six SUHs at two concentration levels (µg/L): Level 1: 65 for CS and MSM; 95 for NS; 125 for FS, PS, and CME; and Level 2: 130 for CS and MSM; 190 for NS; 250 for FS, PS, and CME in order to evaluate the repeatability of the method. Under the optimal experimental conditions, the sample was extracted in triplicate and injected in duplicate the same day. Using the same concentration levels for three consecutive days, following a single extraction and injection, the same water sample that was used to evaluate repeatability was used to validate the method reproducibility. As seen in Table 2, the method precisions for intra-day and inter-day ranged from 3.01 to 8.36 and 2.92 to 9.78, respectively, in terms of relative standard deviations (RSDs%). The experimental findings of precision that were attained for repeatability and reproducibility (RSDs < 9.8%), demonstrating a low variability extraction method.

Sensitivity

The sensitivity of the method guaranteed the detection and confirmation of SUHs residues in water found at levels below or above the limits of detection (LODs). The limits of detection (LOD) and quantification (LOQ) were determined as the lowest concentration yielding a signal-to-noise (S/N) ratio of 3 and 10, respectively. The results are given in Table 2, showing that the LODs ranged from 0.8 to 1.5 ng mL^{-1} while LOQs from 1.9 to 5.1 ng mL^{-1}.

Table 2. Analytical figures of merit for the DLLME technique combined with HPLC-DAD for sulfonyl urea herbicide residues under study.

Analyte	LR[a]	LOD[a]	LOQ[a]	r^2	Level 1		Level 2	
					RSD[b]	**RSD[c]**	**RSD[b]**	**RSD[c]**
NS	4.5-750	1.1	3.2	0.9986	4.12	8.69	4.34	7.86
MSM	3-500	0.8	2.5	0.9979	5.31	9.78	7.90	7.17
CS	3-500	0.9	2.8	0.9994	4.7	5.22	5.15	2.92
FS	6-1000	1.5	5.1	0.9992	8.36	7.35	8.13	6.12
PS	2.5-500	0.8	1.9	0.9986	3.01	9.25	3.72	6.91
CME	6-750	1.2	3.8	0.9998	4.15	9.21	4.80	7.83

Level 1: 65 µg/L for CS and MSM; 95 µg/L for NS; 125 µg/L for FS, PS, and CME.

Level 2: 130 µg/L for CS and MSM; 190 µg/L for NS; 250 µg/L for FS, PS, and CME.

LR[a], LOD[a] and LOQ[a] (ng/mL); RSD[b]: intra-day precision (n = 6); RSD[c]: inter-day precision (n = 9).

Applications of the DLLME method to real environmental water samples

Four environmental water samples, including underground, river, lake, and tape water, were used to validate the proposed methods accuracy. When the unspiked sample was analyzed to see if the six chosen target SUHs were found or not, none of the tested water samples exhibited signals corresponding to values above the LODs. The results that were obtained could mean that the tested samples were either free of pesticide residues or contained levels that were below the detectable limits. The accuracy of the presented method was assessed using the average relative recovery (RR%) of each sample spiked at two concentration levels and extracted in triplicate (Table 3). To evaluate the accuracy of the methods, relative recovery was determined using peak area ratio of each analyte after extraction with proposed method to peak area of the standard solution for the same two concentration levels (Table 3). For the analyzed water samples, RR% at two concentration levels ranged from 84.3 to 101.7, with %RSD < 9.8. The results obtained for recovery were indicating that the matrices of water samples have no significant effect on performance of the proposed method.

Table 3. Relative recovery (RR) values of the proposed method in environmental water samples.

Water sample	Spiked level	Analytes					
		NS	**MSM**	**CS**	**FS**	**PS**	**CME**
		%RR (%RSD, n = 3)					
Underground	Level 1	87.9(7.4)	89.2(9.3)	86.6(9.2)	89.6(8.9)	86.4(5.2)	88.9(9.8)
	Level 2	92.5(8.4)	87.2(3.1)	95.0(4.2)	97.1(4.3)	91.6(4.7)	91.1(5.3)
River	Level 1	89.0(6.5)	86.3(4.9)	89.9(6.6)	90.2(5.0)	89.1(4.7)	85.9(8.8)
	Level 2	84.3(5.4)	88.7(3.5)	90.8(1.6)	89.3(2.15)	84.4 (8.3)	93.1(4.2)
Tape	Level 1	96.0(3.1)	91.9(2.2)	94.6(4.2)	94.8(5.3)	95.1(2.7)	96.1(2.6)
	Level 2	96.2(2.5)	93.7(5.5)	92.5(6.7)	87.5(6.4)	97.9(1.2)	97.5(7.4)
Lake	Level 1	93.49(4.9)	89.3(3.5)	90.9(6.4)	101.7(3.6)	93.7(4.7)	92.4(2.9)
	Level 2	89.1(2.4)	92.4(3.1)	94.8(2.8)	99.1(6.6)	92.3(5.6)	93.1(4.1)

Level 1: 65 µg/L for CS and MSM; 95 µg/L for NS; 125 µg/L for FS, PS, and CME.

Level 2: 130 µg/L for CS and MSM; 190 µg/L for NS; 250 µg/L for FS, PS, and CME.

The chromatograms of the target SUH residues in the river water sample before and after spiking at concentration (level 2) utilized for precision study using the developed methods are displayed in Figure 9. By comparing the peaks of the fortified and blank samples, selectivity was evaluated. These chromatograms clearly show the absence of the chromatographic peak from co-extracted components and that they are well resolved for all analytes, indicating a high level of selectivity for the target pesticides at their retention time. As a result, the given chromatogram confirms the developed DLLME techniques selectivity. Similar characteristics were present in other water samples tested for this study.

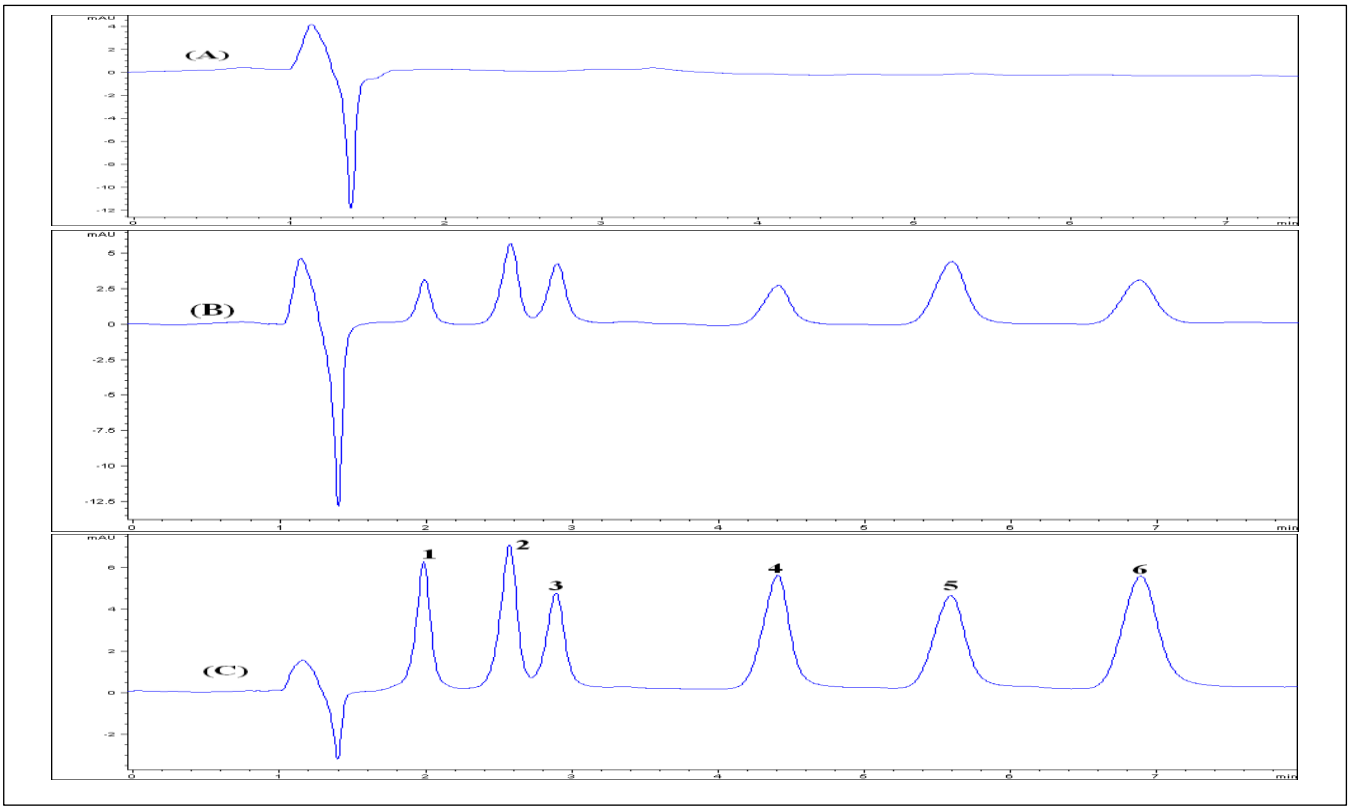


Figure *9.* Typical chromatograms of blank (A) and spiked (B) river water sample, standard (C) at concentrations 130 µg/L for CS and MSM; 190 µg/L for NS; 250 µg/L for FS, PS, and CME; Extraction conditions: the same as those indicated in Fig.8; Peaks identification: 1, niclosulfuron 2, metsulfuron-methyl 3, chiorsulfuron 4, flazasulfuron 5, prosulfuron 6, chlorimuron-ethyl.

Comparison of HD-DLLME with other reported extraction methods

To evaluate the performance of the present method, i.e., DLLME–HPLC–DAD for extraction, enrichment, and determination of SUH residues, it was compared with other methods reported in the literature for extraction of the same herbicide class [14, 30, 36, 43, 46–49] and the results are shown in Table 4. As can be seen, in terms of the LODs, precisions, and accuracy of the present method were better than or comparable to most of other methods applied for extraction of SUH residues from different and same type of matrices, i.e., water sample for current method. Although the LOD of magnetic solid phase extraction (MSPE) method [48] using magnetic multi-walled carbon nanotubes (mag-MWCNTs)

as adsorbents was lower, the synthesis steps of mag-MWCNTs were very complicated, expensive and a large number of organic solvents were consumed for elution and preparation. There may also be the problem of facing sample carryover effects which leads to false postive results [36, 48, 49]. The proposed DLLME is simple and unlike the SPE method, it does not require multi-steps conditioning, washing, loading and elution [20, 21, 48]. In addition, the proposed method is found to use simpler equipment, exhibits a wider linear range, integrated pretreatment and preconcentration in a single step, utilizes micro level amount of organic solvents; which would make the procedure process easier, efficient, quicker, and more environmentally friendly.

Table 4**.** Comparison of the proposed method with other methods applied for the extraction and determination of sulfonylurea herbicides.

Extraction Method	**Determination method**	**Matrix**	**Linear range (ng/mL)**	**LOD (ng/mL)**	**RSD**	**Recovery**	**Ref.**
SD-SFO-DLLME	HPLC-UV	Water and soil	5-1000	0.24-2.7	2.73-12.4	76-107	[14]
IL-DLLME-DSPE	HPLC-DAD	Soymilk	7.8-500	1.53-2.32	1.12-6.48	82-119	[36]
VA-IL-DLLME	HPLC-DAD	Wine	11-450	3.2-6.6	1-6.9	79-106	[43]
CPE	HPLC-UV	Water, soil and rice	4-2000	0.8-1.2	0.4-7.8	82-95	[46]
QuEChERS	HPLC-DAD	Soymilk	200-5000	20-40	<15	61-108	[47]
MSPE	HPLC-DAD	Water	0.05-5	0.01-0.04	2-12.9	77–107	[48]
MMF-SPME	HPLC-DAD	Water	0.1-200	1.8-18.0	1.2-9.9	71-119	[49]
DLLME	HPLC-DAD	Water	2.5-1000	0.8-1.5	2.9-9.8	84-102	This work

CPE; cloud point extraction.

VA-IL-DLLME; vortex-assisted ionic liquid DLLME.

QuEChERS; Quick, easy, cheap, effective, rugged and safe.

MSPE; magnetic solid-phase extraction.

SD-SFO-DLLME; solvent based demulsifications surface floating organic droplet DLLME.

MMF-SPME; multiple monolithic fiber solid phase microextraction.

IL-DLLME-DSPE; ionic liquid based DLLME followed by dispersive solid phase extraction.

CONCLUSIONS

The most widely utilized six SUHs were extracted and quantitatively determined at trace levels in environmental water samples using an HD-DLLME analytical approach that was developed and optimized in the current investigation. All of the experimental attributes taken into account in the study were optimized. No matrix interferences were co-extracted during the procedure used to extract trace level herbicides from water samples such as tap, river, lake, and underground, and weren't seen in the analysis at their respective retention times. It was found that the analytes could be extracted from samples using the optimized experimental approach with only a little amount of extraction and dispersive solvents and a short equilibrium time. The suggested method combines the benefits of a short analysis time, simplicity, low consumption of organic solvent, sensitivity, and cost effectiveness as well as a high level of linearity over a broad range of analyte concentrations. As a result, the HD-DLLME analytical technique might be thought of as a good option for selective and sensitive extraction and practical assessment of SUH residues in environmental water samples in routine laboratory analysis.

ACKNOWLEDGMENTS

The authors are thankful to the Department of Chemistry of Addis Ababa University (AAU), for the laboratory facilities. Material supports from the International Science Program (ISP) through the previous project entitled “Trace Level Pollutants Analyses (ETH:04)”is greatly acknowledged. Habtamu Bekele thanks Oromia Regional State Education Bureau for sponsoring his PhD studies.

REFERENCES

1. Ghobadi, M.; Yamini, Y.; Ebrahimpour, B. Extraction and determination of sulfonylurea herbicides in water and soil samples by using ultrasound-assisted surfactant-enhanced emulsification microextraction and analysis by high performance liquid chromatography. *Ecotoxicol Environ Saf.* **2015**, 112, 68-73.
2. Sarmah, A.K.; Sabadie, J. Hydrolysis of sulfonylurea herbicides in soils and aqueous solutions: A review. *J Agric Food Chem.* **2002**, 50, 6253-6265.
3. Fenoll, J.; Hellín, P.; Sabater, P.; Flores, P.; Navarro, S. Trace analysis of sulfonylurea herbicides in water samples by solid phase extraction and liquid chromatography-tandem mass spectrometry. *Talanta* **2012**, 101, 273-282.
4. You, X.; Chen, L. Analysis of sulfonylurea herbicides in grain samples using molecularly imprinted polymers on the surface of magnetic carbon nanotubes by extraction coupled with HPLC. *Anal. Methods* **2016**, 8, 1003-1012.
5. Kaczyński, P.; Łozowicka, B. One-step QuEChERS-based approach to extraction and cleanup in multiresidue analysis of sulfonylurea herbicides in cereals by liquid chromatography–tandem mass spectrometry. *Food Anal Methods* **2017**, 10, 147-160.
6. Perreau, F.; Bados, P.; Kerhoas, L.; Nélieu, S.; Einhorn, J. Trace analysis of sulfonylurea herbicides and their metabolites in water using a combination of off-line or on-line solid phase extraction and liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem.* **2007**, 388, 1265-1273.
7. Fang, G.; Chen, J.; Wang, J.; He, J.; Wang, S. N-Methylimidazolium ionic liquid functionalized silica as a sorbent for selective solid phase extraction of 12 sulfonylurea herbicides in environmental water and soil samples. *J Chromatogr A* **2010**, 1217, 1567-1574.
8. Fang, G.; Wang, X.; Wang, S. Multiwalled carbon nanotubes as solid phase extraction adsorbents for simultaneous determination of seven sulfonylurea herbicides in environmental water by LC-MS/MS. *Chromatographia* **2010**, 72, 403-409.
9. Shamsipur, M.; Yazdanfar, N.; Ghambarian, M. Combination of solid phase extraction with dispersive liquid-liquid microextraction followed by GC-MS for determination of pesticide residues from water, milk, honey and fruit juice. *Food Chem.* **2016**, 204, 289-297.

10. Pastor-Belda, M.; Garrido, I.; Campillo, N; Viñas, P.; Hellín, P.; Pilar Flores, J.F. Combination of solvent extractants for dispersive liquid-liquid microextraction of fungicides from water and fruit samples. *Food Chem.* **2017**, 233, 69-76.
11. Berrada, H.; Font, G.; Moltó, J.C. Influence of the solvent on the gas chromatographic behaviour of urea herbicides. *Chromatographia* **2001**, 54, 253-262.
12. Gure, A.; Lara, F.J.; Megersa, N.; Garc, A.M.; Olmo-iruela, M. Hollow-fiber liquid phase microextraction combined with capillary HPLC for the selective determination of six sulfonylurea herbicides in environmental waters. *J Sep Sci.* **2013**, 36, 3395-3401.
13. Gallitzendo, R.; Koch, D.; Ku, M. Simultaneous determination of 12 sulfonylurea herbicides in drinking water after SPE by LC-DAD. *Chromatographia* **2011**, 73, 813-816.
14. Yan, L; Jing, Z.; Lin, R.; Yongxin, L.; Xiaoli, Z. Application of solvent demulsification-dispersive liquid-liquid microextraction based on solidification of floating organic drop coupled with high perfomence liquid chromatography in determination of sulfonylurea herbicides in water and soil. *J Braz Chem Soc.* **2016**, 1-8.
15. Wang, D.; Lu, Z.; Guan, X.; Yang, M.O.; Guo, H.; Yang, Z. Magnetic polydopamine modified with choline-based deep eutectic solvent for the magnetic solid phase extraction of sulfonylurea herbicides in water samples. *J of Chromatographic Sci.* **2021**, 59, 95-102.
16. Li, C.; Lu, A.; Wang, J.; Li, J.; Ping, H.; Luan, Y.; Chen, J.; Ha, X. Determination of five sulfonylurea herbicides in environmental waters and soil by ultra high performance liquid chromatography with tandem mass spectrometry after extraction using graphene. *J Sep Sci.* **2014**, 37(24), 3714-3721.
17. Song, N.; Guo, M.; Liu, Y.; Shi, L. Rapid residue analysis of sulfonylurea herbicides in surface water : Methodology and residue findings in eastern tiaoxi river of china. *J Mater Sci Chem Eng.* **2016**, 4, 41-50.
18. Zhao, L.; Szakas, T.; Churley, M.; Lucas, D. Multi-class multi-residue analysis of pesticides in edible oils by gas chromatography-tandem mass spectrometry using liquid-liquid extraction and enhanced matrix removal lipid cartridge cleanup. *J Chromatogr A* **2019**, 1584, 1-12.
19. Timofeeva, I.; Shishov, A.; Kanashina, D.; Dzema, D.; Bulatov, A. On-line in-syringe sugaring-out liquid-liquid extraction coupled with HPLC-MS/MS for the determination of pesticides in fruit and berry juices. *Talanta* **2017**, 167, 761-767.

20. Han, F.; Zhou, D.B.; Song, W.; Hu, Y.Y.; Lv, Y.N.; Ding, L.; Zheng, P.; Jia, X.Y.; Zhang, L.; Deng, X.J. Computational design and synthesis of molecular imprinted polymers for selective solid phase extraction of sulfonylurea herbicides. *J Chromatogr A* **2021**, 1651, 462321.
21. Samsidar, A.; Shaarani, S. A review of extraction, analytical and advanced methods for determination of pesticides in environment and foodstuffs. *Trends Food Sci Technol.* **2018**, 71, 188-201.
22. Bekele, H.; Megersa, N. Green analytical method based on salt assisted graphene oxide dispersive solid phase extraction of symmetrical triazine herbicides in environmental water samples for liquid chromatographic determination. *Int. J. Environ. Anal. Chem.* **2023**, 1-17.
23. Liu, Q.; Li, Z.; Wei, L.; Chen, X.; Xu, Y.; Zhao, J. Fast dispersive liquid – liquid microextraction of pesticides in water based on a thermo - switchable deep eutectic solvent. *Environ Chem Lett.* **2022**, 20,2271-2276.
24. Viñas, P.; Martínez-Castillo, N.; Campillo, N.; Hernández-Córdoba, M. Liquid-liquid microextraction methods based on ultrasound-assisted emulsification and single-drop coupled to gas chromatography-mass spectrometry for determining strobilurin and oxazole fungicides in juices and fruits. *J Chromatogr A* **2010**, 1217, 6569-6577.
25. Megersa, N. Hollow fiber-liquid phase microextraction for trace enrichment of the residues of atrazine and its major degradation products from environmental water and human urine samples. *Anal Methods* **2015**, 7, 9940-9948.
26. Yang, J. A novel method for the determination of trace sulfonylurea herbicides by introducing a hybrid stationary phase to common capillary. *Adsorpt Sci Technol.* **2017**, 35, 372-385.
27. Chen, L.; Wu, J.; Huang, X. Multiple monolithic fibers modified with a molecularly imprinted polymer for solid phase microextraction of sulfonylurea herbicides based on boron-nitrogen interaction. *Microchim Acta.* **2019**, 186, 470.
28. Rezaee, M.; Assadi, Y.; Milani Hosseini, M.R.; Aghae, E.; Ahmadi, F.; Berijani, S. Determination of organic compounds in water using dispersive liquid-liquid microextraction. *J Chromatogr A* **2006**, 1116, 1-9.
29. Wang, Y.L.; You, L.Q.; Mei, Y.W.; Liu, J.P.; He, L.J. Benzyl functionalizedionic liquid as new extraction solvent of dispersive liquid-liquid microextraction for enrichment of organophosphorus pesticides and aromatic compounds. *Chinese J Anal Chem*. **2016**, 44, 942-949.

30. Wu, Q.H.; Li, Y.P.; Li, C.; Wu, C.X.; Liu, Z.M.; Hou, Y.X.; Wang, Z. Dispersive liquid – liquid microextraction combined with high performance liquid chromatography-DAD detection for the determination of sulfonylurea herbicides in water samples. *Int J Environ Anal Chem.* **2010,** 90, 891-902.

31. Bekele, H.; Megersa, N. Fast surface floating organic droplets based dispersive liquid-liquid microextraction for trace enrichment of multiclass pesticide residues from different fruit juice samples followed by high performance liquid chromatography – diode array detection analy. *Sep Sci plus* **2023**, 6(8), 1-12.

32. Huang, X.; Du, Z.; Wu, B.; Jia, L.; Wang, X.; Jing, X. Dispersive liquid – liquid microextraction based on the solidification of floating organic droplets for HPLC determination of three strobilurin fungicides in cereals. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* **2020**, 37(8), 1279-1288.

33. Cheng, J.; Zhou, Y.; Zuo, M.; Dai, L.; Guo, X. Application of dispersive liquid-liquid microextraction and reversed phase-high performance liquid chromatography for the determination of two fungicides in environmental water samples. *Int J Environ Anal Chem.* **2010**, 90, 845-855.

34. Carvalho, R.R.R.; Rodriguez, M.D.V.R.; Franco, E.S.; Beltrame, F.; Pereira, A.L.; Santos, V.S.; Araujo, W.; Rocha, B.A.; Rodrigues, J.L. DLLME-SFO-GC-MS procedure for the determination of 10 organochlorine pesticides in water and remediation using magnetite nanoparticles. *Environ Sci Pollut Res.* **2020**, 27(36), 45336-45348.

35. Beshana, S.; Hussen, A.; Leta, S.; Kaneta, T. Dispersive liquid–liquid microextraction coupled with microfluidic paper-based analytical device for the determination of organophosphate and carbamate pesticides in the water sample. *Anal Sci.* **2022**, 38, 1359-1367.

36. Li, J.; Xu, W.; Wang, X.; Wu, F.; Wang, L.; Feng, J.; Wang, Z.; Zhang, H. Ionic liquid-based dispersive liquid–liquid microextraction followed by dispersive solid phase extraction coupled with HPLC-DAD for the determination of sulfonylurea herbicides in soymilk samples. *J Liq Chromatogr Relat Technol.* **2021**, 44(13-14), 663-673.

37. Carbonell-rozas, L.; García-campaña. A.M.; Lara, F.J.; Iruela, O. Capillary liquid chromatography as an effective method for the determination of seven neonicotinoid residues in honey samples. *J Sep Sci.* **2020**, 43, 3847–3855.

38. Tian, H.; Bai, X.; Xu, J. Simultaneous determination of simazine, cyanazine, and atrazine in honey samples by dispersive liquid–liquid microextraction combined with high-performance liquid chromatography. *J Sep Sci.* **2017**, 40, 3882-3888.
39. Tursen, J.; Yang, T.; Bai, L.; Li, D.; Tan, R. Determination of imidacloprid and acetamiprid in bottled juice by a new DLLME-HPLC. *Environ Sci Pollut Res.* **2021**, 28, 50867-50877.
40. Farajzadeh, M.A.; Pirmohamadlou, A.; Sattari Dabbagh, M. Extraction and preconcentration of some pesticides in vegetable and fruit juice samples using SA@$CaCO_3$ sorbent combined with dispersive liquid–liquid microextraction. *Food Anal Methods* **2021**, 14, 2395-2407.
41. Ma, L.; Wang, Y.; Li, H.; Peng, F.; Qiu, B.; Yang, Z. Development of QuEChERS-DLLME method for determination of neonicotinoid pesticide residues in grains by liquid chromatography-tandem mass spectrometry. *Food Chem.* **2020**, 331, 127190.
42. Hammad, S.F.; Abdallah, I.A.; Bedair, A.; Mansour, F.R. Salting out induced liquid–liquid microextraction for alogliptin benzoate determination in human plasma by HPLC/UV. *BMC Chem*. **2021**, 15(1), 1-10.
43. Gure, A.; Lara, F.J.; García-Campaña, A.M.; Megersa, N.; Del Olmo-Iruela, M. Vortex-assisted ionic liquid dispersive liquid–liquid microextraction for the determination of sulfonylurea herbicides in wine samples by capillary high performance liquid chromatography. *Food Chem.* **2015**, 170, 348-353.
44. Gure, A. Lara, F.J.; Moreno-González, D.; Megersa, N.; Del Olmo-Iruela, M.; García-Campaña, A.M. Salting out assisted liquid–liquid extraction combined with capillary HPLC for the determination of sulfonylurea herbicides in environmental water and banana juice samples. *Talanta* **2014**, 127, 51-58.
45. Sazali, N.H.; Alshishani, A.; Saad, B.; Chew, K.Y.; Chong, M.M. Salting out assisted liquid–liquid extraction coupled with high performance liquid chromatography for the determination of vitamin D_3 in milk samples. *R Soc Open Sci.* **2019**, 6, 1-8.
46. Wu, Y.J.; Fu, X.W.; Yang, H. Cloud point extraction with triton X-114 for separation of metsulfuron-methyl, chlorsulfuron, and bensulfuron-methyl from water, soil, and rice and analysis by high performance liquid chromatography. *Arch Environ Contam Toxicol.* **2011**, 61, 359-67.

47. Rejczak, T.; Tuzimski, T. Simple, cost-effective and sensitive liquid chromatography diode array detector method for simultaneous determination of eight sulfonylurea herbicides in soya milk samples. *J Chromatogr A* **2016**, 1473, 56-65.
48. Ma, J.; Jiang, L.; Wu, G.; Xia, Y.; Lu, W.; Li, J. Determination of six sulfonylurea herbicides in environmental water samples by magnetic solid phase extraction using multi-walled carbon nanotubes as adsorbents coupled with high performance liquid chromatography. *J Chromatogr A* **2016**, 1446, 12-20.
49. Pei, M.; Zhu, X.; Huang, X. Mixed functional monomers based monolithic adsorbent for the effective extraction of sulfonylurea herbicides in water and soil samples. *J Chromatogr A* **2018**, 1531, 13-21.

6. GENERAL CONCLUSIONS AND RECOMMENDATIONS

In this thesis, four environmentally friendly and miniaturized sample preparation techniques were developed for preconcentration, extraction and quantification of trace level selected pesticide residues in environmental water and food samples.

In the first paper, a GO nanomaterial was synthesized and used successfully as SPE sorbent. The developed analytical method based on the SA-GO-DSPE combined with HPLC–DAD was applied for selective sample extraction and simultaneous quantitative determination of the most commonly used four triazine pesticides at trace level in water samples. When the method applied to the extraction of triazines from real water samples such as tap, spring, underground and river water; no matrix interferences were co-extracted and observed in the analysis with liquid chromatographic separation. The sorbent used as nanoparticle was observed to possess high adsorption capacity and rapid adsorption rates. In the experimental procedure, it was realized that only low amount of sorbent can be used and also short equilibrium time was required to extract the analytes from the contaminated samples. Hence, the method has demonstrated the advantages of short analysis time, simplicity, easy operation and low organic solvent consumption; which implies the method to be in good agreement with the principles of Green Chemistry, suggesting the established method combined with HPLC–DAD could be successfully applied as a reliable alternative for preconcentration, extraction, and determination of triazine pesticides in environmental water samples and other trace organic pollutants in routine laboratory analysis.

In the second part of research work, a DLLME-SFOD analytical method coupled with HPLC−DAD instrumentation was developed and optimized for selective and simultaneous extraction and quantitative determination of the most commonly used seven multiclass pesticides at trace levels in fruit juice samples. The extraction technique was optimized for all the experimental parameters considered in the study. No matrix interferences were co-extracted or seen in analysis at the corresponding retention times when the method was used to extract trace level pesticide residues from fruit juice samples such as orange, grape, guava, apple, and pineapple. The extraction solvents used in the current extraction method are more eco-friendly compared to other extraction solvents utilized in other reported studies that used toxic halogenated organic solvents. In the optimized experimental procedure, it was realized that only microliter quantities

of extraction and disperser solvents have been used and also short equilibrium time was required to extract the analytes from samples. The suggested technique combines the advantages of a quick analysis time, simplicity, minimal organic solvent consumption, sensitivity, and cost-effectiveness as well as a high level of linearity over a wide range of analyte concentrations. Therefore, the trace level enrichment of multiclass pesticides using the DLLME-SFOD analytical technique could be considered as a good alternative for selective and sensitive extraction and convenient assessment of multiclass pesticide residues in fruit juice samples for routine laboratory analysis.

In the third research work, SALLE–HPLC–DAD analytical technique, that is simple, fast, and green, was developed and optimized for routine monitoring and quantitative determination of seven multiclass pesticide residues with a wide range of physicochemical properties, including methidathion, atrazine, azoxystrobin, cyanazine, carbrayl, thiamethoxam, and propazine in the samples of raw and pasteurized milk. Comparing to more traditional extraction techniques like LLE and SPE, this method uses a significantly smaller extraction solvent and sample volume. For all of the experimental factors taken into account during the investigation, the approach was optimized utilizing univariate methods. The optimized method offers sufficient accuracy, precision, linearity, and sensitivity under the optimum extraction conditions in short extraction time. No matrix interferences were co-extracted or seen in the analysis at their respective retention times while this method was being used to extract trace level pesticides from milk samples. Comparing to other reported research methods that used hazardous halogenated organic solvents as extraction solvents, the extraction solvents used in the current extraction approach are more environmentally benign. As a result, the trace level enrichment of multiclass pesticides using the SALLE analytical technique could be considered as a good alternative for selective and sensitive extraction and practical assessment of multiclass pesticide residues in milk as well as enrichment of other trace compounds in complex samples in routine laboratory analysis.

In the fourth research work, the most widely utilized six SUHs were extracted and quantitatively determined at trace levels in environmental water samples using HD-DLLME analytical approach that was developed and optimized in this study. All of the experimental attributes taken into account were also optimized. No matrix interferences were co-extracted during the procedure used to extract trace level herbicides from water samples such as tap, river, lake, and underground, and

weren't seen in the analysis at their respective retention times. It was found that the analytes could be extracted from samples using the optimized experimental approach with only microliter volume of extraction and disperser solvents and a short equilibrium time. The suggested method combines the benefits of a short analysis time, simplicity, low consumption of organic solvent, sensitivity, and cost effectiveness as well as a high level of linearity over a broad range of analyte concentrations. As a result, the HD-DLLME analytical technique could be taken as a good option for selective and sensitive extraction and practical assessment of SUH residues in environmental water samples in routine laboratory analysis.

The analytical sample preparation method, SA-GO-DSPE coupled with HPLC-DAD was developed and optimized for the first time using salt assisted prominent GO as dispersed sorbent for sensitive and selective enrichment, extraction and determination of most commonly used selected triazine herbicides in environmental water samples. In addition, to the best of our knowledge, this is the first instance in which DLLME-SFOD and SALLE combined with HPLC-DAD were developed for simultaneous extraction and determination of selected multiclass pesticide residues in different fruit juices and cow milk samples, respectively. Furthermore, HD-DLLME sample preparation method, which was scarce in published literature works reported was developed for enrichment and extraction of most commonly used selected SUHs from environmental water samples. The developed microextraction methods were found simple, fast, selective, inexpensive and environmentally safe alternative approach with simple instrumentation to be used in laboratories of developing country.

Researchers are working to create genetically modified crops in modern agriculture that can make their own pesticides or show resistance to pests or broad-spectrum herbicides. Biological pesticide control methods are also recommended wherever possible to apply. The use of chemicals and their damaging effects on the environment and human health could be minimized by using this new pest management strategy and leaving buffer zones around sensitive regions while applying pesticides.

The developed techniques may be suggested for monitoring these pesticide contaminants in samples of water and food. To improve selectivity and sensitivity of the process and apply it to more complex matrices, synthesis of functionalized graphene nanomaterials for use as sorbents for

solid phase extraction to increase selectivity and sensitivity method and to apply for more complex matrices are generally advised. The proposed methods can also be improved in terms of using even less toxic solvents, further miniaturization for enriching better, extraction efficiencies, selectivity, and sensitivity, as well as full automation and on-line coupling capability with analytical instruments. Additionally, the developed methods have been expanded to a large number of other intensive agricultural zones in the country.