

## ورتکس یاری شده با دانسیته پایین بر اساس میکرواستخراج مایع-مایع پخشی دنبال شده با کروماتوگرافی مایع با کارایی بالا برای تعیین سه آفت کش باقیمانده در نمونه‌های آب

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تاریخ دریافت: ۱۰ اسفند ۱۳۹۵ تاریخ پذیرش: ۲۷ اسفند ۱۳۹۵

## Vortex-Assisted Low Density Based Dispersive Liquid-Liquid Microextraction Followed by High Performance Liquid Chromatography for Determination of Three Pesticides Residues from Water Samples

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Received: 28 February 2017

Accepted: 17 March 2017

### چکیده

در این مطالعه، ورتکس یاری شده با دانسیته پایین بر اساس میکرواستخراج مایع-مایع پخشی و دنبال شده با کروماتوگرافی مایع با کارایی بالا توسط آشکارساز ماوراء بنفش برای تعیین آفت کش های کلرفلورنل-متیل، کلرنتوینفس و دی آزینون در نمونه‌های آب‌های محیطی بکار گرفته شد. پارامترهای مختلف مؤثر بر راندمان استخراج، شامل نوع و حجم حلال استخراجی و پخش کننده pH نمونه، اثر نمک، زمان سانتیفریژ و ورتکس بررسی گردید و شرایط بهینه بدست آمد. تحت شرایط بهینه، منحنی‌های کالیبراسیون در محدوده غلظتی ۸/۵-۱۰۰ و ۳/۵-۱۰۰ و ۳۶/۵-۶۰۰ نانوگرم بر میلی‌لیتر برای سه آفت کش نامبرده با ضریب همبستگی ( $r^2$ ) برابر با ۰/۹۹۳ یا بهتر خطی بودند. حدود تشخیص و تعیین آنالیت‌ها بر اساس ۳ و ۱۰ برابر نسبت سیگنال به نویز به ترتیب در محدوده ۱۱-۰/۹ و ۳/۱-۳۶/۸ نانوگرم بر میلی‌لیتر محاسبه گردید. روش پیشنهادی بطور موفقیت آمیزی برای آنالیز نمونه‌های آب بکار برده شد. بازیابی‌های نسبی (RR%) با اضافه کردن دو سطح غلظت در محدوده ۷۹-۱۰۸٪ و با انحراف استاندارد‌های نسبی (RSD%) در رنج ۹/۱-۹/۹٪ مطالعه گردید. نتایج این مطالعه اثبات می‌کند که روش پیشنهادی برای استخراج یا پیش تغلیظ سه آفت کش قبل از اندازه‌گیری کمی توسط دستگاه HPLC-UV/V مؤثر می‌باشد.

### واژه‌های کلیدی

کروماتوگرافی مایع با کارایی بالا؛ ورتکس یاری شده؛ دانسیته پایین؛ میکرواستخراج مایع-مایع پخشی؛ آفت کش؛ آب محیطی.

### Abstract

In this study, vortex-assisted low density based dispersive liquid-liquid microextraction followed by high performance liquid chromatography with ultraviolet detector has been developed for the determination of three pesticides including chlorflurenol-methyl, chlorfenvinphos, and diazinon from environmental water samples. Different parameters influencing the extraction efficiency such as the type and volume of extraction and disperser solvent, sample pH, salt addition as well as vortex and centrifugation time were investigated and the optimal conditions were obtained. Under the optimum conditions, the calibration curves were linear in the concentration range of 8.5–100, 3.1–100 and 36.5–600 ng/mL for chlorflurenol-Methyl, chlorfenvinphos and diazinon, respectively, with coefficient of determination ( $r^2$ ) of 0.993 or better. The limits of detection and quantification of the analytes, which were determined at 3 and 10 signal-to-noise ratio (S/N) ranged from 0.9–11 and 3.1–36.8 ng/mL, respectively. The proposed method has been successfully applied to the analysis of real water samples. The relative recoveries (%RR) studied at two spiking concentration levels were ranging from 76–108%, with the corresponding relative standard deviation (%RSD) ranging from 1.9–9.9%. The results of study demonstrated that the proposed method is efficient for extraction and/or preconcentration of the three pesticides prior to quantitative determination utilizing HPLC-UV/V is instrument.

### Keywords

High Performance Liquid Chromatography; Vortex-Assisted; Low Density; Dispersive Liquid-Liquid Microextraction; Pesticides; Environmental Water.

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## 1. INTRODUCTION

Pesticides are pure or mixture of substances intended for preventing, repelling, or killing of pests. "Pesticide" is a general term; it includes insecticides, herbicides, rodenticides, fungicides, miticides, as well as wood preservatives, disinfectants, products that control algae, etc. [1-3]. Based up on their chemical structures and functional groups pesticides can also be classified into a number of chemical classes including organophosphorus, organochlorine, carbamate, and pyrethroids and so on [3-4].

The widespread uses of pesticides for agricultural and non-agricultural purposes have resulted in the presence of their residues in various environmental matrices, such as soil, water (surface water and groundwater) and air [5]. Most pesticides are characterized by pronounced persistence against chemical or biological degradation, high environmental mobility, strong tendency for bioaccumulation in human and animal tissues and significant impacts on the health of human beings [4, 5]. From the total applied pesticides, below 0.1% could reach the target pests and the rest proportion might be disseminated into other environmental compartments via various mechanisms including leaching, agricultural or urban runoff, drift, etc. [5, 6].

The presence of pesticide residues in environment including water ecosystem has been identified to cause risks on crops, aquatic plants and microorganisms and human being [5]. According to World Health Organization (WHO) estimation, every year about three million people could die because of pesticides poisoning [7]. This indicates that contamination of pesticides in different environmental system is a serious problem, necessitating the regular monitoring of their levels in water ecosystem.

Various analytical techniques have been used for the determinations of pesticide residues from water samples. These techniques include liquid chromatography (LC) with ultraviolet (UV) detector [8], diode array detectors (DAD) [9-12] and mass spectrometry detector (MS) [13, 14, 15]; gas chromatography (GC) with detectors such as flame ionization detection (FID) [5, 16], flame photometric detector (FPD) [17, 18], nitrogen-phosphorus detection (NPD) [18], MS [16, 20, 21, 22], and tandem MS (MS/MS) [23]; micellar electrokinetic chromatography with ultraviolet/visible detector (MEKC-UV) [24] and laser-induced fluorescence detection [25]. Bhadekar and coworkers [26] reviewed different analytical techniques that have been used for analysis of pesticide residues from water samples.

On the other hand, due to the low concentration of pesticide residues in real sample matrices, rigorous and time-consuming sample preparation step is required prior to their quantitative analysis. Sample preparation involves isolation (extraction) and/or preconcentration of analytes from real sample matrices prior to quantitative determinations [27]. Thus, in the last three decades, several, new, novel modified sample preparation methods involving superior advantages including simplicity, quickness, low cost, high recoveries, high preconcentration factor, minimal toxic organic solvent consumption and reduced wastes, over the traditional methods: liquid-liquid extraction (LLE) [20] and solid phase extraction (SPE) [21, 22] have been developed and utilized for analysis of pesticide residues in various matrices including environmental waters [12, 20, 28-30].

Among the proposed new and/or modified sample preparation methods, dispersive liquid-liquid microextraction (DLLME) is one method. It was first reported in 2006 by Rezaee and coworkers [20] and since then, it has shown enormous application for extraction and/or preconcentration of various organic and inorganic pollutants from different matrices [23, 24, 29-31]. The DLLME method employs a ternary solvent system: aqueous sample, extraction solvent, and disperser solvent. The method involves a rapid injection of a mixture of extraction and disperser solvents, in preset proportion, into the aqueous sample to induce a cloudy suspension consisting of fine droplets of extraction solvent dispersed in the aqueous sample. After extracting, the fine droplets containing the analytes are separated by centrifugation for the subsequent analysis [20, 28-31].

On the other hand, many modifications have been made on the method, to further simplify the procedure and above all to avoid the use of highly toxic higher density halogenated organic solvents than water as extraction solvent [9, 11, 31]. To this end, many attempts have been made to use low density organic solvents than water [9, 11, 30, 31] and/or ionic liquids [28, 30, 32], which are generally less toxicity and environmental safe. Low density organic solvents based DLLME (LD-DLLME) method have been utilized for extraction and preconcentration of various classes of pesticides including *s*-triazine herbicides [11], sulfonylurea and organophosphorus pesticides [9], organophosphorus pesticides [16, 33], organochlorine pesticides [34, 35] and carbamates pesticides [36-38] from various aqueous samples. However, no work has been reported on the use of

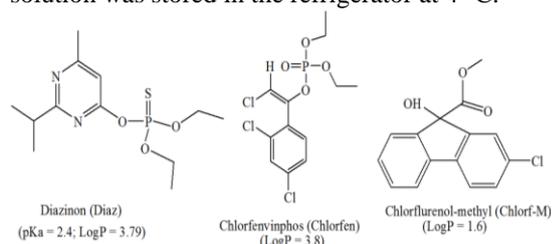
vortex assisted (VA-LD-DLLME) method in combination with HPLC-UV/Vis for the analysis of --- three pesticide residues including chlorflurenol-methyl (Chlorf-M), chlorfenvinphos (Chlorf) and diazinon (Diaz) from environmental water samples. Therefore, in this study, VA-LD-DLLME has been proposed for extraction and preconcentration of these pesticide residues from environmental water samples. Vortex agitation is used to accelerate dispersion of the extraction solvent into the aqueous sample and subsequently, enhancing the extraction efficiency of the method [32]. Finally, the extract has been separated and quantitative analysed utilizing HPLC-UV/Vis.

## 2. EXPERIMENTAL

### 2.1. Chemicals and reagents

All chemicals and reagents used were analytical grade and solvents were HPLC grade. The organic solvents cyclohexane, 1-octanol, acetone, ethanol, hexane and toluene as well as chemical and reagents such as phosphoric acid ( $H_3PO_4$ ), sodium hydroxide (NaOH), hydrochloric acid (HCl), and sodium chloride (NaCl) were obtained from BDH Chemicals Ltd (Poole, England). Ultrapure water obtained after purification utilizing Mill-Q water purification system, (Millipore, Bedford, France), was used throughout the work. Whatman® filter paper (grade 1 and size 8.5 cm) obtained from Whatman International Ltd (Maidstone, England) was used for filtration of the water samples.

Analytical standards of diazinon (Diaz), chlorfenvinphos (Chlorfen) and chlorflurenol-methyl (Chlorf-M) were purchased from Sigma Aldrich (St. Louis, MO, USA). The chemical structures, common names, abbreviations, the  $pK_a$  and  $\log P$  of the target pesticides are given in Fig. 1. Stock solutions containing 1000 mg/L of each pesticide were prepared by dissolving an accurately weighed amount of each pesticide in methanol and stored in refrigerator below 4 °C. Intermediate working standard solution containing 10 mg/L for Chlorfen and Chlorf-M and 60 mg/L for Diaz, respectively, was also prepared in methanol and then, the prepared solution was stored in the refrigerator at 4 °C.



**Fig. 1.** Chemical structure and common names of the target pesticides.

### 2.2. Instruments and equipment

Separation and quantification were performed using PerkinElmer HPLC quaternary solvent systems (Shelton, USA) equipped with Flexar solvent manager, Flexar LC autosampler, pump, column compartment and UV/V is detector. Chromatographic separations were carried out using a Brownlee Analytical  $C_{18}$  column (150 x 4.6 i.d., particle size 3  $\mu m$ ) obtained from PerkinElmer, Inc. (Winter Street, Waltham, USA). Sample processing and data acquisitions were performed using chromera software (4.1.16396). A Vortex mixer model FB15024 obtained from Fisher scientific (Kunststal 21, 9900 Eeklo, Belgium), pH meter from Hanan instruments (Póvoa de Varzim, Portugal) and ultrasonic water bath obtained from Elma Schmidbauer GmbH (Singen, Germany) were utilized during sample preparation.

### 2.3. Chromatography conditions

Reversed phase chromatographic separation was performed using earlier mentioned column. A binary mobile phase consisting of solvent A (ultrapure water) and solvent C (acetonitrile) with isocratic elution at the ratio of (water: acetonitrile, 40:60, v/v) was utilized throughout the analysis. Chromatographic separation was performed at the flow rate of 1 mL/min, 25°C column temperature, 10  $\mu L$  injection volume and 254 nm UV monitoring wavelength.

### 2.4. Water samples

Three different environmental water samples were collected in polyvinylchloride (PVC) bottles from different localities of Jimma town, Jimma Ethiopia. River water was collected from Adari river Seto Kebele; groundwater was collected from Jiren Kebele; and tap water was collected from Jimma university chemistry department research laboratory. The collected water samples were then stored in dark below 4°C until the time of analysis, without any pretreatment.

### 2.5. VA-LD-DLLME procedures

River water and groundwater samples were filtered utilizing Whatman filter paper before introducing into VA-LD-DLLME. After adjusting pH to 5 utilizing acetate buffers, 10 mL of each water sample, was taken into a 15 mL centrifuge tube and 10 % (w/v) NaCl was then added. Subsequently, a mixture containing 75  $\mu L$  cyclohexane and 1000  $\mu L$  acetonitrile as extraction and disperser solvents, respectively, was rapidly injected into the sample solution and then, the cloudy solution was formed. Thereafter, the sample solution was vortexed for about 10 s to enhance the homogeneous distribution of the

cloudy suspension into the sample solution and thus, ensure rapid transfer of the analyte from the aqueous phase to the organic phase (fine droplets). The mixture was then centrifuged at 4,000 rpm for 4 min to enhance phase separation of the fine droplets of organic phase. The fine droplets were then collected at top surface of the aqueous sample. Afterward, 50  $\mu\text{L}$  of the upper organic phase was carefully taken via micro pipette and was then transferred into a 150  $\mu\text{L}$  insert vial and was then placed in 2 mL autosampler vial. Eventually, to make compatible with the HPLC instrument, the extract was diluted to the total volume of 150  $\mu\text{L}$  by adding methanol and then, 10  $\mu\text{L}$  was injected into the instrument.

### 3. RESULT AND DISCUSSION

#### 3.1. Optimization of chromatographic condition

To obtain rapid chromatographic separation of the target analytes, series of preliminary experiments were performed by using two different binary solvents systems; i.e., using water, with either methanol or acetonitrile. A binary mobile phase composition comprising, water and acetonitrile, at the ratio of 40:60, (v/v) exhibited better performance for separation of the target analytes in less than 10 min and thus selected for further experiments. Chromatographic separation was performed at the flow rate of 1 mL/min, column temperature at 25°C and monitoring wavelength of 254 nm.

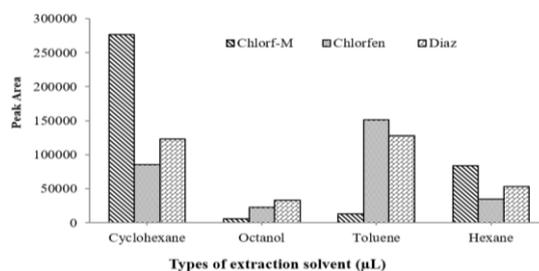
#### 3.2. Optimization of VA-LD-DLLME

In order to achieve optimum conditions for VA-LD-DLLME method, the influence of various experimental parameters such as type and volume of the extraction and disperser solvents, pH of the sample solution, salt addition, and centrifuge as well as vortex agitation time were investigated. All experiments were performed in replicates and then, averages of the peak areas were used to evaluate the extraction efficiency of the different experimental parameters.

##### 3.2.1. Effect of the type of extraction solvent

Selection of an appropriate extraction solvent is crucial to develop efficient VA-LD-DLLME method [9, 31]. In this study, the extraction solvents were selected based on their low solubility in water, low toxicity, lower density than water, high extraction capability for the interested analytes and good chromatographic behavior [11, 33-35] Accordingly, four solvents including 1-octanol (density,  $d = 0.827 \text{ g/mL}$ ), toluene ( $d = 0.865 \text{ g/mL}$ ), n-hexane ( $d = 0.659 \text{ g/mL}$ ) and cyclohexane ( $d = 0.779 \text{ g/mL}$ ) were evaluated as candidate of extraction solvent. As can be seen in Fig. 2 the highest peak areas were

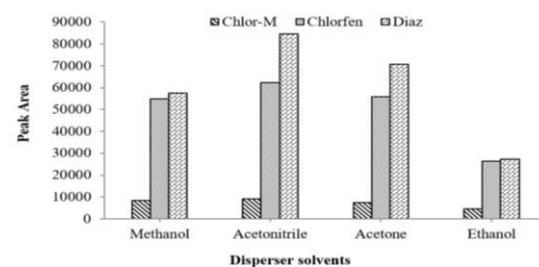
obtained for all compounds when cyclohexane was used and thus it was chosen as the extraction solvent in further studies.



**Fig. 2.** Effect of type of extraction solvent. Extraction conditions: sample volume (10 mL), volume of the extraction solvent (75  $\mu\text{L}$ ), acetonitrile (1000  $\mu\text{L}$ ) as disperser solvent, vortex time (10 s), centrifugal time (4 min at 4000 rpm).

##### 3.2.2. Effect of the type of disperser solvent

Selection of disperser solvent is based on its miscibility with both organic and aqueous sample phases [29, 31]. Disperser solvent usually causes the extraction solvent to be break down into fine droplets, which could evenly be distributed into the aqueous sample, resulting in an enhanced contact area between extraction solvent and aqueous solution, thus facilitate the extraction efficiency of the target analyte into the organic phase [9, 20]. In this study, four solvents including methanol, acetonitrile, ethanol and acetone were investigated as a disperser. The obtained results are shown in Fig. 3. As can be seen from the figure, acetonitrile displayed the highest peak areas for all target analytes and thus, it was chosen as a disperser solvent in subsequent experiments.

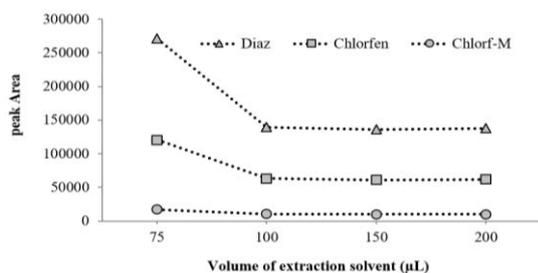


**Fig. 3.** Effect of the types of disperser solvent. Extraction conditions: cyclohexane as extraction solvent (75  $\mu\text{L}$ ), volume of disperser solvent (1000  $\mu\text{L}$ ), other conditions similar with Fig. 3.1.

##### 3.2.3. Effect of volume of extraction solvent

In DLLME procedure, the volume of extraction solvent is another crucial parameter influencing the extraction efficiency of the method [16, 23]. In the present study different volumes of the extraction solvent, ranging from 50–200  $\mu\text{L}$  were evaluated by mixing with a fixed volume, i.e., 1000  $\mu\text{L}$ , of the disperser solvent. It was observed

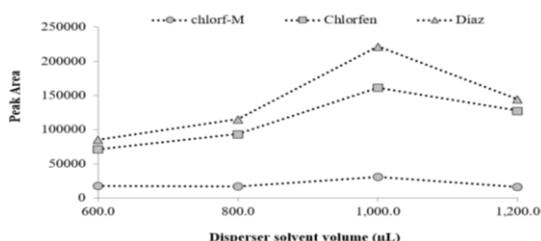
that the volume of organic phase floated on the top of the aqueous phase increases with increasing of volume of the extraction solvent. But, with the 50  $\mu\text{L}$  insignificant phase separation was exhibited. As can be seen from Fig. 4, the obtained peak areas of the target analytes decreased as the volume of the extraction solvent increased due to dilution effect [9] and thus, 75  $\mu\text{L}$  of cyclohexane (the extraction solvent) was selected as the optimum in further studies.



**Fig. 4.** Effect of the volume of extraction solvent. Extraction conditions: cyclohexane as extraction solvent, acetonitrile as disperser solvent (1000  $\mu\text{L}$ ), other conditions similar with Fig. 3.1.

### 3.2.4. Effect of volume of disperser solvent

The volume of disperser solvent can affect the solubility of extraction solvent in aqueous phase and thus affecting extraction efficiency of the method [31]. To acquire the optimum volume, experiments were carried out with different volumes of acetonitrile ranging from 600-1200  $\mu\text{L}$  containing 75  $\mu\text{L}$  of cyclohexane. The peak area of disperser solvent increased with the increasing of the volume of acetonitrile up to 1000  $\mu\text{L}$  then slightly decreased at higher the volume of acetonitrile as can be observed from Fig. 5. At low volume of acetonitrile cloudy solution is not well formed, so that the extraction efficiency of target analytes from the sample solution were low, while at higher volumes, the solubility of the target analytes in aqueous solution increases and thus, resulted in decreased peak areas [9]. Therefore, 1000  $\mu\text{L}$  volumes of acetonitrile were selected in the further experiments.



**Fig. 5.** Effect of the volume of disperser solvent. Extraction conditions: cyclohexane as extraction solvent (75  $\mu\text{L}$ ), acetonitrile as disperser solvent, other conditions similar with Fig. 3.1.

### 3.2.5. Effect of the pH

The pH of water sample has a significant effect on the extraction efficiency of the analytes. The pH of water sample affects the existing degree of ionization of a target analyte in aqueous solution, which has an effect on the extraction capacity of the extraction solvent. To evaluate this parameter, experiments were carried out with the pH of the aqueous samples ranging from 2.0 to 7.0. However, the pH value above 7.0 was not studied since the pesticides might undergo degradation under the alkaline condition. It was observed, though the effect of the sample pH has less effect on the peak areas of the target analytes relatively the highest peak area was obtained at pH 5.0 and thus, pH 5 was selected for further studies.

### 3.2.6. Salt addition

The addition of small amount of salt, such as NaCl, into the sample solution induces salting-out effect, decreasing the solubility of the extraction solvent as well as the analytes in the aqueous solution and thus, accelerate phase separation [16, 23]. The effect of salt addition was investigated by adding NaCl for 0-15% (w/v). It was observed that the peak areas of the target analytes increases with the increase of salt concentration up to 10% and then, started to slightly decline. Therefore, 10% was chosen as the optimum concentration of salt.

### 3.2.7. Effect of vortex agitation and centrifugation times

Vortex agitation is generally employed in DLLME procedure in order to accelerate dispersion of extraction solvent into aqueous solution, thereby increasing extraction efficiency [39, 40]. The influence vortex agitation time on the extraction efficiency of the method was evaluated from 0-30 s at rotation speed of 1800 rpm. It was observed that the peak area of the target analytes increase with agitation time up to 10 s and then, leveled off on further increase in vortex agitation time, so 10 s was chosen as optimum vortex time.

The influence of centrifugation time on the extraction efficiency of the method was investigated from 1-5 min, at the speed of 4000 rpm. In LD-DLLME centrifugation is used to accumulate the extraction phase at the top of the aqueous phase [31]. It was observed that the peak areas of the target analytes exhibited slight increase up to 4 min and then, became constant on further increase of centrifugation time. Thus, 4 min was selected as optimal centrifugation time for the subsequent studies.

## 3.3. Method Validation

### 3.3.1. Calibration curves and analytical performance characteristics

The developed VA-LD-DLLME method was validated by constructing matrix-matched calibration curves utilizing the target analyte free river water sample as a representative matrix. The

calibration curves were constructed for six various concentration levels. Using the optimum conditions, each concentration level was extracted in duplicate and each extract was then injected in duplicate. Thereafter, the calibration curves were obtained by plotting peak areas (instrumental response) as a function of the analytes concentrations. For all analytes, wide linearity ranges with coefficient of determinations ( $r^2$ ) of 0.993 or better were achieved. The limits of detection (LOD) and quantification (LOQ) determined as the smallest concentrations that give 3 and 10 times a signal-to-noise ratio (S/N), were obtained in the range of 0.9-11 and 3.1-36.8 ng/mL, respectively. Details of the figures of merits of the proposed method are presented in Table 1.

**Table 1.** Figures of merits of the proposed VA-LD-DLLME.

Analyte	Linear range, ng/mL	$r^2$	LOD, ng/mL	LOQ, ng/mL
Chlorf-M	8.5 -100	0.993	2.6	8.5
Chlorfen	3.1 -100	0.994	0.9	3.1
Diaz	36.8 -600	0.994	11	36.8

### 3.3.2. Precision study

The precision of the proposed VA-LD-DLLME method was investigated in terms of intra- and inter-day precisions. Inter-day precision was studied by extracting the spiked river water samples at two concentration levels. Each concentration level was extracted as well as also injected in duplicate on the same day. Inter-day precision of the method was also evaluated for five consecutive days at both concentration levels earlier used for intra-day precision studies. As can be observed from Table 2 the RSD of both intra- and inter-day precisions were below 7.0%, indicating the proposed method has acceptable precisions for the analysis of the target analytes from water and related samples [41].

**Table 2.** Intra-day (n = 4) and inter-day (n = 10) precisions of the proposed method (RSD, %) for the spiked water samples.

Analyte	Intra-day		Inter-day	
	Level 1	Level 2	Level 1	Level 2
Chlorf-M	6.4	8.1	1.7	9.6
Chlorfen	5.2	6.8	1.3	2.4
Diaz	1.9	2.7	6.6	2.8

Level 1: 10 ng/mL for Chlorf-M, Chlorfen, and 60 ng/mL for Diaz.

Level 2; 60 ng/mL for Chlorf-M, Chlorfen, and 360 ng/mL for Diaz.

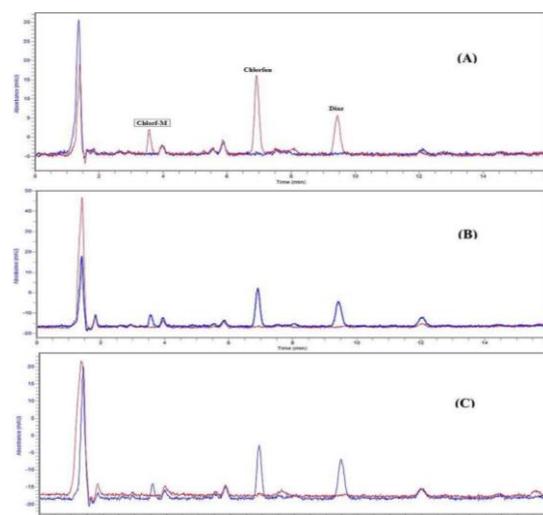
### 3.3.3. Analysis of real samples and recovery studies

The applicability of the developed method was evaluated by performing relative recovery (%RR) studies utilizing three different types of environmental waters comprising tap water, river water and ground water. %RR studies were performed by spiking each water sample at two concentration levels earlier employed for precision studies (Table 2). For each

concentration level, two samples were extracted using the proposed method and each extract were then injected in duplicates. For each water sample, blank samples were also extracted and analyzed by the proposed method. However, none of the target analytes was detected in studied water samples as can be seen in Fig. 6. %RR of the analytes was determined by comparing the peak area of the spiked water samples with that of the peak area obtained for the spiked ultrapure water sample. The obtained %RRs of the target analytes with their %RSDs for each water samples are presented in Table 3. The observed %RR of the analytes were in the range of 76–108 %, with %RSD varying from 1.2–9.9 %, indicating the proposed method has acceptable relative recoveries and precisions for the analysis of the target pesticides in different environmental water samples [41].

**Table 3.** Relative recoveries, %RR (%RSD, n = 4) of the method for the spiked, river water, tap and groundwater samples.

Analytes	River water		Tap water		Groundwater	
	Level 1	Level 2	Level 1	Level 2	Level 1	Level 2
Chlorf-M	102(4.3)	81(5.4)	101(3.3)	80(9.9)	101(3.7)	87(9.6)
Chlorfen	80(9.2)	76(8.1)	95(1.2)	99(1.9)	108(7.7)	98(9.7)
Diaz	100(6.5)	83(6.3)	82(9.4)	93(6.4)	85(1.3)	89(5.1)



**Fig. 6.** Typical chromatograms of the target analytes in the three environmental water samples: (A) Groundwater blank and spiked, (B) Tap water blank and spiked and (C) River water blank and spiked.

#### 4. CONCLUSIONS

In this study, a new analytical method based on VA-LD-DLLME combined with HPLC-UV has been developed for the analysis of three pesticides (Chlorf-M, Chlorfen and Diaz) from environmental water samples. Various parameters influencing the extraction efficiency of the methods were seriously studied and the optimum conditions were established. Utilizing the optimal conditions, the proposed VA-LD-DLLME method exhibited its usefulness for the determination of the target pesticides with acceptable analytical performances, precision and recoveries. In conclusion, the obtained results demonstrated that the developed method could be effectively used as a simple alternative for rapid extraction, preconcentration and determination of the three target pesticides in water samples and other related matrices.

#### ACKNOWLEDGMENTS

Authors are grateful to the Department of Chemistry of the Jimma University for providing the laboratory facilities. Financial support for some of the work was obtained from school of graduate studies of Jimma University.

#### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. The article also does not contain any studies with human or animal subjects.

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