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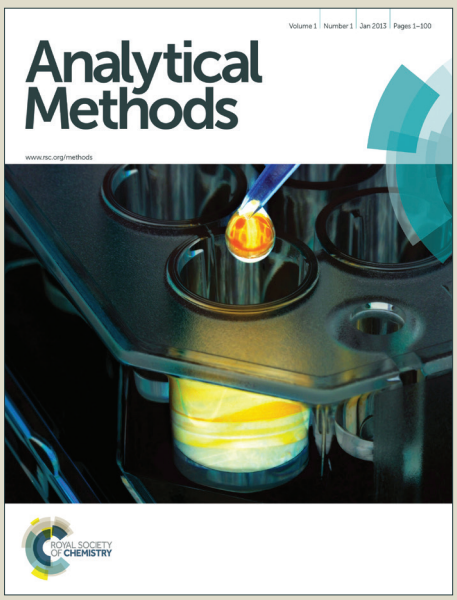

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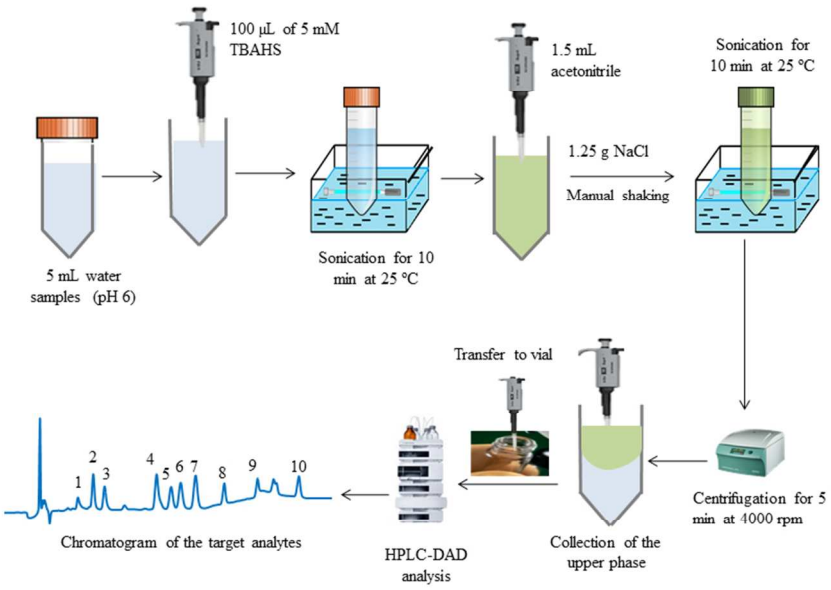

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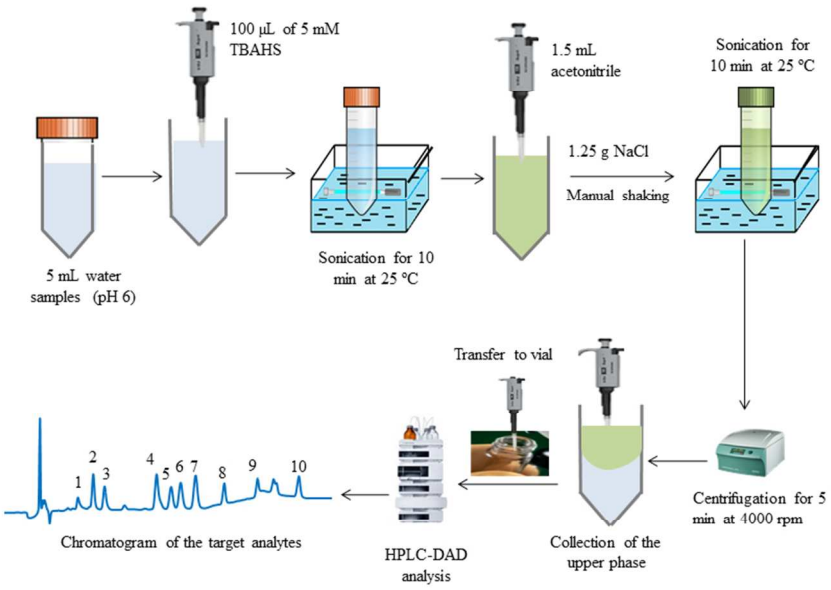

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Ion-pair assisted liquid-liquid extraction for selective separation and analysis of multiclass pesticide residues in environmental waters

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Abstract

A new ion-pair assisted liquid-liquid extraction (IPA-LLE) in combination with high performance liquid chromatography-diode array detector (HPLC-DAD) has been proposed for the determination of ten multiresidue pesticides; six sulfonylurea (SU) and four organophosphorus (OP) pesticides, in environmental waters. In the IPA-LLE procedure, the ion-pairing reagent tetrabutylammonium hydrogen sulfate (TBAHS) and the organic solvent, acetonitrile, were used for extraction of the target analytes. Various parameters influencing the extraction efficiency such as the type, composition and volume of ion-pair (IP), volume of acetonitrile, sample pH, type and composition of the salt and effect of sonication time were studied and optimal conditions were established. Under the optimum conditions, the limits of detection (LOD) and quantification (LOQ) of the proposed method were in the ranges of 0.5–3.0 μg L^{-1} and 1.8–10.0 μg L^{-1}, respectively, and calibration curves were linear within the range of 1.8–450 μg L^{-1}, with coefficient of determination of 0.993 or better. Intra- and inter-day precision studies, expressed as relative standard deviations (%RSDs), at three concentration levels, were in the range of 0.4–9.4%. The relative recoveries of the spiked environmental water samples were in the range of 73–105%, except for NS in lake water. The results of the study revealed that the developed method involves efficient sample preparation allowing the preconcentration of analytes, followed by the use of HPLC-DAD for quantitative analysis.

1. Introduction

The use of synthetic organic pesticides has increased over the past decades in order to control and destroy pests. Although their uses increased agricultural productivity, their extensive use has resulted in contamination of various environmental components including water resources.[1–3] Consequently, pesticides of different chemical structures and properties including sulfonylurea (SU) and organophosphorus (OP) pesticides have been detected in ground and surface waters, with quantities exceeding the maximum residue levels (MRLs) set by several legislative authorities.[4–6]

SU pesticides are one of the most commonly used classes of pesticides for control of grasses and broad-leafed weed species in a variety of crops and vegetables.[7] They are efficient at low application doses and the second most commonly used kind of herbicides in the recent years, after glyphosates, and more than 30 products have been commercialized.[8,9] On the other hand, OP pesticides constitute the most widely used insecticides today, in modern agriculture worldwide. Though, they are considered safer than organohalides pesticides, they are known to be neurotoxic to humans. They are strong inhibitors of cholinesterase enzymes that function as neurotransmitters. OP pesticides are also highly absorbed by inhalation, ingestion and skin penetration.[10]

The residues of pesticides can enter into the ground and surface waters through leaching and runoff from soil and thus can potentially affect the human health.[11] Due to their occurrence in trace levels and complexity of the environmental water samples, analysis of these compounds require the use of selective and efficient sample preparation methods that can simultaneously extract and preconcentrate trace levels of the target analytes prior to their instrumental determinations, while rejecting the matrix interferents to significantly reduce their effects. Despite their enormous drawbacks, such as the use of large quantities of sample and hazardous organic solvents, traditional sample preparation methods such as liquid-liquid extraction (LLE)[12] and solid-phase extraction (SPE)[13,14] are still the most commonly used methods for quantitative extraction of multiresidue pesticides from environmental waters.

However, simultaneous extraction and preconcentration of multiresidue pesticides that constitute polar and nonpolar compounds into nonpolar organic solvents is a challenging experimental task because of the higher solubility of the polar analytes in aqueous solution. LLE can be modified to

simultaneously extract polar pesticides like SUs and nonpolar compounds such as OP pesticides using ion-pair assisted liquid-liquid extraction (IPA-LLE). IP extraction is the method of choice for selective extraction of polar (i.e., acidic/basic) compounds from aqueous samples into organic phase with the aid of counter ions, comprising different hydrophobicity as ion-pairing reagents.[15] It is usually performed by adding an IP reagent to the sample solution containing ions of the target analytes, to form IP complexes that possess higher partition coefficients than the target analytes and thus enhancing their transfer into the extractant (organic) phase.[16]

IP extraction has long been used in combination with various sample preparation techniques such as SPE,[17–21] solid-phase microextraction (SPME),[22] single-drop microextraction (SDM),[23–26] hollow-fiber liquid-phase microextraction (HF-LPME),[27,28] supported liquid membrane, SLM[29] and LLE using water immiscible organic (extraction) solvent such as chloroform[30–32] and water-miscible organic (extraction) solvent including ethyl acetate,[33] acetonitrile,[34,35] acetone[36] and methanol[37] for selective extractions of various ionizable organic compounds, including acidic/basic pesticides. In general, when water miscible organic solvents such as acetonitrile, acetone, ethyl acetate, etc are used as extraction solvent, in LLE, formation of a two-phase system occurs upon addition of appropriate quantity of a salt; a phase separation process that occurs due to salt addition is referred to as "*salt induced phase separation*".[38,39]

Though, IPA-LLE has been used for quantitative determination of several polar organic compounds and metal analytes, to date the method has not been reported for simultaneous residual analysis of polar SU and nonpolar OP pesticides in any matrix. Therefore, in the current study, a novel sample preparation technique based IPA-LLE using water miscible extraction solvent, acetonitrile, in combination with HPLC-DAD for quantitative determination of ten multiresidue pesticides, including six polar SUs; chlorimuron-ethyl (CSE), metsulfuron-methyl (MSM), nicosulfuron (NS), prosulfuron (PS), rimsulfuron (RS) and triflusulfuron-methyl (TSM) and four nonpolar OP compounds; chloropyrifos (Chlor), diazinon (Diaz), fenitrothion (Fen), methidathion (Meth) in environmental water samples has been proposed. Various parameters affecting the extraction efficiency of the technique as well as experimental parameters influencing the separation efficiencies of the target analytes were investigated so as to establish the optimum conditions. The applicability of the proposed analytical technique has also been experimentally

evaluated by applying to different environmental water samples of varying chemical compositions.

2. Experimental

2.1. Chemicals and reagents

Analytical standards of chloropyrifos (Chlor), diazinon (Diaz), fenitrothion (Fen), methidathion (Meth), metsulfuron-methyl (MSM), nicosulfuron (NS), prosulfuron (PS), rimsulfuron (RS) and triflusulfuron-methyl (TSM) were purchased from Sigma Aldrich (St. Louis, MO, USA). Chlorimuron-ethyl (CSE) was obtained from ChemServiceInc (West Chester, USA). The chemical structures, common names, abbreviations and the pK_a of the target pesticides are given in Fig. 1. Individual stock standard solutions, 1000 mg L^{-1}, and intermediate working solution containing 20 mg $L^{-1}$of each analyte, were prepared in acetonitrile. All solutions were stored under refrigeration below 4 ºC.

Fig. 1 here

All chemicals used in this sudy were of analytical grade reagents and the solvents were HPLC grade. Tetrabutylammonium hydrogensulfate, TBAHS ($C_{16}H_{37}NO_2S$); tetrabutylammonium iodide, TBAI ($C_{16}H_{36}NI$) and hydrochloric acid (HCl) were obtained from Sigma-Aldrich (St. Louris, MO, USA). Potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen orthophosphate, anhydrous (K_2HPO_4), sodium hydroxide (NaOH), sodium chloride (NaCl), acetic acid glacial (AHOc), ammonium sulphate ($(NH_4)_2SO_4$) and magnesium sulphate ($MgSO_4$) were purchased from BDH chemical Ltd (Poole, England). Methanol and acetonitrile were obtained from Carlo Erba Reactifs-SDS, (Val de Revil, France) and Ashland chemical (S. Giuliano MI, Italy), respectively. Ultrapure water was obtained after purification with double distiller, A8000 Aquatron water Still, (Bibby Scientific Ltd, Staffordshire, United Kingdom) and deionizer Thermo Scientific Barnstead E-Pure™, (Thermo Fisher Scientific Inc., Italy) was used throughout the work. Aqueous mobile phase was filtered under vacuum through cellulose acetate Millipore filter membrane, S-Pak, black with white grid surface, 0.45 µm x 47 mm obtained from Sigma-Aldrich (St. Louris, MO, USA). 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.

2.2. Instruments and equipment

High performance liquid chromatographic (HPLC) system, Agilent 1200 series (Agilent Technologies, Waldbronn, Germany) equipped with quaternary pump (flow range 0.2–10 mL min^{-1}), vacuum degasser, thermostatted autosampler and column compartments as well as multiple wavelength diode array detector (DAD) was used for the sample analysis. Chromatographic separations were carried out using Nucleosil C_{18} column (250 x 4.6 mm I. D., 5 µm particle size and 100 Å pore size) from Phenomenex (Torrence, CA, USA). Sample processing and data acquisitions were performed using ChemStation B.02.01-SR1.

Measurement of pH was performed using a pH meter; Adwa, model 1020 Adwa Hungary Kft. (Szeged, Hungary). An ultrasonic heater, Dacon®, Dacon laboratories, Ltd (St. Hove, East Sussex), centrifuge, Model 800 Jiangsu Zhenji insturuments Co., Ltd. (Jiangsu, China) and 15 mL centrifuge tube, corning incorporated, (Corning, NY, Mexico) were used for sample preparation.

2.3. Chromatographic conditions

Chromatographic separations were carried out based on the findings of our earlier collaborative works,[40] with some modifications, using Nucleosil C_{18} column. A binary mobile phase comprising of solvent A (ultrapure water) and solvent B (acetonitrile), both containing 0.01% HAOc with a gradient program of 45–45% B (10 min), 45–75% B (3 min) and 75% B (9 min) was used throuhgout the analysis. Prior to the sample/extract injection, the HPLC column was equilibrated with the initial composition of the mobile phase for 10 min. Analysis was performed with the mobile phase flow rate of 1 mL min^{-1}, column temperature 30 ^{o}C, injection volume 15 µL and monitoring wavelength of 236 nm.

2.4. Water samples

Three different types of environmental water samples were collected in PVC bottle from different localities in Ethiopia: groundwater from Mekanisa, Addis Ababa, and river and lake waters both from the Oromia Region State; Teji River and Hora Lake, Eastern and South Western Shoa Zones, respectively. The water samples were stored at 4 ºC in the dark prior to analysis, without any pretreatment.

2.5. Ion-pair assisted salting-out liquid-liquid extraction (IPA-LLE) procedure

All water samples were filtered Whatman filter paper and then subjected to the ion-paired extraction procedure. Thereafter, 5 mL water sample containing 10 mmol L^{-1} phosphate buffer (pH 6) was transferred into a 15-mL falcon tube and was then spiked with appropriate concentrations of the target pesticides and kept to stand for about 20 min for equilibration. After addition of 100 µL of 5 mmol L^{-1} TBAHS, the content was sonicated for 10 min at 25 °C, to enhance the ion-pairing processes. Then, 1.5 mL acetonitrile and 1.25 g (i.e., 25%, m/v) NaCl was added and the resulting mixture was shaken manually until the salt was completely dissolved. The content was then sonicated for 20 min at 25 °C. In order to achieve efficient phase separation, the content was centrifuged for 5 min at 4000 rpm. Finally, the upper phase was carefully withdrawn using micropipette and transferred to the autosampler vial for the subsequent injection to the HPLC system. Schematic description for the entire experimental procedure is provided in Fig. 2.

Fig. 2 here

3. Results and discussion

3.1. Optimization of HPLC conditions

Efficient analyte resolution in the chromatographic analysis is the preliminary experimental exercises usually considered. This could be achieved by preforming series of experiments while varying the composition of the mobile phases. Accordingly, the binary mobile phases, used in the present study; namely, water (solvent A) and acetonitrile (solvent B), both containing 0.01% HOAc (v/v) were varied. In order to obtain efficient separation, in a reasonable analysis time, various gradient programs were investigated at a flow rate of 1 mL min^{-1} and finally, the gradient program comprising 45–55% B (10 min), 55–75% B (3 min) and 75% B (9 min) exhibted good chromatographic separation for all the target compounds in 22 min. Prior to the next sample injection, the HPLC system was re-equilibrated with the initial composition of the mobile phase for 10 min.

The effect of injection volume was investigated over the range of 10–30 µL. It was observed that the peak areas of all the target analytes increased with the injection volume, though above 15 µL

some peaks including that of PS, Meth, CSE and TSM were observed broader and their resolutions were also not satisfactory. Thus, injection volume of 15 µL was selected as a compromise between the sensitivity and peak resolution. The effect of mobile phase flow rate was also evaluated in the range of 0.8–1.2 mL min^{-1}. It was observed that both the retention times and peak widths of all the target pesticides were lowered with increasing the flow rates and besides resolution between Meth and CSE decreased for the higher flow rates and thus a flow rate of 1 mL min^{-1} was chosen as the optimum throughout the analysis. The column temperature was also evaluated in the range of 25–35 ºC and no significant change was observed in the studied temperature range. Thus, the column temperature was set at 30 ºC to analyse all the pesticides at 236 nm DAD monitoring wavelength throughout this work.

3.2. Optimization of IPA-LLE

In the present study, IPA-LLE using a cationic ion-pairing reagent and a water miscible organic solvent, acetonitrile, has been proposed for simultaneous extraction and preconcentration of polar SU and non polar OP pesticides from environmental waters. The IP based LLE procedure involves two equilibrium processes including the formation of IPs in the sample solution and distribution of the IPs between the aqueous and organic phases.[27] The transfer rate of IPs from the aqueous to organic phase, i.e., the IP extraction rate, is dependent on diffusive mass transfer rather than the rate of the chemical reaction between the cations and anions.[28,33] However, the diffusive mass transfer rate of the analytes can be influenced by the parameters such as the type, concentration and volume of the ion-pairing reagent, volume of the organic solvent (acetonitrile), pH of the sample, type and composition of the salt as well as sonication time. Therefore, these parameters were thoroughly investigated in order to establish the optimal conditions that could provide the highest extraction efficiency. All experiments were performed in triplicate by spiking 5 mL ultrapure water (containing 10 mmol L^{-1} phosphate buffer) with 100 µg L^{-1} of all the target pesticides. The average peak areas of the replicate analyses were considered to evaluate the effect of the experimental parameters, on the extraction performance of the method.

3.2.1. Selection of the organic ion-pair

The polar/ionizable pesticides considered in this study are weak acids with pK_a values ranging from 2.4-4.55 (Fig. 1). In the aqueous solution, these compounds exist predominantly in neutral form at pH values below the pK_a and in an anionic form at the pH above their pK_a values.[8] However, in a neutral/alkaline aqueous solution they exist in anionic forms and thus exhibit poor extraction efficiency in LLE. The use of cationic ion-pairing reagent results in the formation of IPs (anion-cation IP association) and subsequently enhances their extractabilities with other nonpolar analytes.[27,28] In this study, extraction performances of two ion-pairing reagents, including TBAHS and TBAI, were evaluated. The observed results demonstrated that similar peak areas were obtained with both TBAHS and TBAI and thus either of them could be used as an ion-pairing reagent. In the present study, TBAHS was chosen and used for further analyses because of sufficient availability.

3.2.2. Effects of TBAHS concentration and volume

The concentration of IP reagent influences distribution of the counter-ions and subsequently the extraction performance of the analytes.[27] As a result, the effect of TBAHS concentrations on the extraction efficiency of the method was studied by varying concentration over the range of 0–20 mmol L^{-1}. As it is evident from Fig. 3, the peak areas of almost all the pesticides increase with increasing the concentration of the IP reagent upto 5 mmol L^{-1} and then decreased up on addition of higher concentrations. The decrease in peak areas of the target analytes at higher concentrations of the TBAHS may be due to the fact that with large excess amount of the TBAHS, the steric hinderance caused by its side chains reduces the IP formation efficiency of the anions of the target analytes in the solution. The same phenomenon was also noted for other similar ionizable compounds.[24,35] Therefore, 5 mmol L^{-1} of TBAHS was selected as optimum for further studies.

Fig. 3 here

The effect of the volume of IP reagent on the extraction efficiency of the presented IP-LLE technique was evaluated by varying the volume of TBAHS over the range of 50–300 µL, at a concentration of 0.5 mmol L^{-1}. It was observed that the extraction efficiency of the target

pesticides were found to increase in the volume range of 50–200 µL and then lowered at higher volumes. The most probable reason associated to the decrease in the extraction efficiency, at higher volume of the IP reagent, might be due to the fact that the target analytes could not properly form IP as a result of steric hindrance from the side chain of the ion-pairing reagent,[35] as described above. Therefore, 100 µL was selected as the optimum volume for subsequent experiments.

3.2.3. Effect of acetonitrile volume

The volume of the organic solvent, acetonitrile, is also another important parameter that could influence the extraction performance of IPA-LLE.[36,39,41] In order to obtain the optimal volume, various volumes of acetonitrile in the range of 0.75–2.5 mL were investigated, while the other experimental parameters were kept constant. As can bee seen in Fig. 4, the extraction efficiency of the target analytes is shown in Fig. 4. It can be seen that the extraction efficiency of all the pesticide compounds increased with the volume of acetonitrile from 0.75–1.5 mL and then decreased upon further increment of acetonitrile. When smaller acetonitrile volumes were used, the boundary between the acetonitrile and the aqueous phases was not clear and this caused collection of the upper organic layer to be difficult, which resulted in inaccurate analysis.[41] On the other hand, the decrease in peak áreas observed at higher volume of acetonitrile may be related to the dilution effect, resulting from higher volume of the organic phase that can be separated after extraction. Therefore, 1.5 mL acetonitrile was observed to be the optimum volume and used in the subsequent experiments.

Fig. 4 here

3.2.4. Effect of the sample pH

In order to achieve good extraction efficiency of the ionizable acidic organic compounds, using IPA-LLE, the analytes should first be transformed to their anionic, i.e., negatively charged forms, which could be achieved by adjusting the pH of the samples.[37] Therefore, the effect of sample pH was studied in the range of 4.0–9.0, in 10 mmol L^{-1} phosphate buffer. The results of the study revealed that pH of the sample have a crucial effect on the extraction efficiency of the studied pesticides (Fig. 5). The peak areas of the target pesticides increased with the rise in pH of the sample solution up to pH 5 and was then remained constant up to pH 6 and then started to

decline upon further increase in pH of the sample. Therefore, pH 6 was selected as optimum for further studies.

Fig. 5. here

3.2.5. Effect of salt type and composition

Generally, salts can cause different degrees of phase separation.[39,42] Therefore, in this study, the effect of three different salts; NaCl, $MgSO_4$ and $(NH_4)_2SO_4$ were evaluated, using 25% (m/v) of each salt, as a potential salting-out reagent.[35,37] It was observed that though $MgSO_4$ and $(NH_4)_2SO_4$ gave better phase separation, the highest peak areas of all the target analytes were obtained when NaCl was used. The observed differences might be attributed to a reduction of preconcentration factors resulting from higher volume of the acetonitrile that could be observed when either $MgSO_4$ or $(NH_4)_2SO_4$ was used as salting-out reagents. Similar finding has also been reported for analysis of penicillin residues.[35] Therefore, NaCl was utilized as the salting-out reagent in all the subsequent studies.

The effect of NaCl concentration on the extraction performances of the target analytes was evaluated by adding different quantities of NaCl, in the range of 1.0–1.75 g (20–35%, m/v), to the aqueous sample solution. The results of the study indicated that the volume of organic phase recovered after extraction increased with the quantity of the salt added. Likewise, the peak areas of the target pesticides also increased with the quantity of the salt and the highest peak areas were obtained for all the compounds at 1.25 g (25%, m/v). However, when higher quantities of NaCl were added, peak areas were started to decline. The decrease in the peak areas of the analytes, at higher concentrations, could be attributed to the dilution effect. Thus, 1.25 g NaCl was used as the optimum quantity of the salt for playing the role of salting out effects, in this study.

3.2.6. Effect of sonication time

Sonication is another important parameter that can greatly influence the extraction efficiency of IPA-LPE.[33] In IPA-LLE procedure sonication is used to promote the formation of IPs in the aqueous sample and also to enhance the transfer of the IPs into the organic phase. In this study, the effect of sonication was investigated by preforming series of experiments: without using

sonication, using sonication only before as well as both before and after addition of acetonitrile and appropriate quantity of the salt. The results of the study revealed that sonication has no significant effect on the extraction efficiency of some of the analytes such as Meth, Diaz, Fen and Chlor, but for the remaining pesticides the peak areas have exhibited the increasing tendency when the system was sonicated twice, demonstrating the importance of sonication both before and after addition of acetonitrile and optimum quantity of the salt.

Then, the effect of sonication time was also studied in the range of 10–40 min total sonication time. The results of the study showed that for those analytes that their extraction performances were affected by sonication, their peak areas were increased with sonication time up to 20 min and then remained constant at longer sonication times and thus 20 min was utilized as the optimum sonication time.

3.3. Evaluation of the proposed method

3.3.1. Calibration curves and analytical performance characteristics

The proposed IPA-LLE combined with HPLC-DAD method was evaluated using matrix-matched calibration curves, which were established in groundwater samples.

The calibration curves were constructed by spiking the mixture of ten pesticides; six SUs and four OPs, at six concentration levels. Each level was extracted in duplicate (experimental replicates) and each extract was then injected in duplicates (instrumental replicates). Calibration curves were obtained by plotting the peak areas as instrumental responses versus the pesticide concentrations. For all the analytes, the coefficients of determination (R^2) of the calibration curves were 0.993 or better, which confirmed a good linearity over the concentration range studied. The limits of detection (LOD) and quantification (LOQ) were considered as the minimum analytes concentrations yielding 3 and 10 times the signal-to-noise (S/N) ratio, respectively. The figures of merit of the proposed method are summarized in Table 1.

Table 1 here

3.3.2. Precision study

The precision of the method was studied in terms of repeatability (intra-day precision) and reproducibility (inter-day precision) applying the optimized conditions to the groundwater samples.

Repeatability of the method was evaluated by extracting spiked groundwater samples at three concentration levels. Each sample was prepared in duplicates (experimental replicates) and was then injected in triplicates (instrumental replicates) on the same day, under the same experimental conditions. Similarly, reproducibility was investigated by extracting one spiked groundwater sample at each of the three concentration levels, utilized for repeatability studies, for five consecutive days and each concentration level was then injected in triplicates. The results of both intra- and inter-day precisions, which were expressed as the relative standard deviations (%RSD) of peak areas, are shown in Table 2. As can be seen, aceptable precisions (less than 10%) were obtained in all cases.

Table 2 here

3.3.3. Applications and recovery studies

The applicability of the proposed method was studied by performing relative recovery studies in three different kinds of environmental waters including groundwater, river water and lake water samples. For the relative recovery (%RR) studies, each kind of these samples was spiked at three concentration levels previously used for precisión studies (see section 3.3.2). At each concentration level, two samples were subjected to the IP-LLE procedure and each of these extracts was then injected in triplicate. In all cases, the blank samples were extracted and analyzed by the proposed method, but, none of the target analytes were detected in these water samples. Howover, in river water simple, PS was not measured because of its poor resolution with the peak appearing from the matrix. Relative recoveries were calculated as the ratio of the peak area of the spiked water samples to the peak area of the spiked ultrapure water sample and the obtained results with their corresponding %RSD for each water samples are shown in Table 3. The obtained %RR with the current method were in the range of 73–105%, with the exception of NS, in the lake wáter, which was around 50%. But in all cases, including NS in lake water, the %RSDs were ranging from 0.4 to 9.4%, indicating that the proposed method has acceptable precisions.[5]

Table 3 here

3.3.4. Selectivity of the analytical technique

Selectivity of the proposed method was also evaluated by comparing the chromatograms of the blank (unspiked) water samples with the corresponding spiked water samples. Fig. 6 shows typical chromatograms of the blank (unspiked) and spiked water samples with the target pesticides. As can be seen from the chromatograms, with the exception of PS in river water, no significant interferences were observed at the retention times of the target analytes. Moreover, the recoveries of NS in lake water were also not satisfactory, which could be attributed to the matrix effect from the sampe. Based on the observed results, in general, the proposed IPA-LLE technique has good selectivity for trace level analysis of the selected pesticides by HPLC-DAD in environmental water samples.

Fig. 6. here

3.3.5. Comparison of the proposed method with other methods

The extraction efficiency of the proposed IPA-LLE procedure has been compared with other recently reported techniques including SPE with various sorbent types such as ionic liquids supported on magnetic nanoparticles (IL-MNPs),[43] silica supported gold nanoparticles (Au-TEOS or Au-NPs),[44] silica supported gold nanoparticles functionalized ionic liquids (Au-NP-IL-Silica),[44] C_{18},[14,44] hollow-fiber liquid-phase microextraction (HF-LPME)[40] and cloud point extraction (CPE)[45] considering parameters such as linearity range, LOD, extraction time, sample volume and %RSD. Details of the comparison are shown in Table 4. In respect to the other techniques, the proposed method utilizes shorter extraction time and smaller sample volume. Moreover, it provides similar or better LODs and linear ranges than the other reported ones. The method also utilizes classical laboratory equipments as well as less toxic organic solvent, which could be accessible in common research laboratories. Based on the experiemental findings and the inferrence from the comparison, general conclusión could be drawn confirming that the proposed method is simple, rapid, cheap and environmentally benign for trace level determination of multiclass pesticide residues in environmental waters and other related matrices.

Table 4 here

4. Conclusion

In the present study, a new analytical method has been proposed for sample preparation and quantitative determination of six SU and four OP pesticides using IPA-LLE in combination with HPLC-DAD from environmental waters. Various parameters affecting the chromatographic separation and the extraction efficiencies of the target analytes were rigorously investigated and the optimum conditions were established. Under the optimum conditions, the proposed ILA-LLE technique demonstrated its usefulness for the determination of all the target analytes with LODs and LOQs varying from (0.5–3.0 μg L^{-1}) and (1.8–10 μg L^{-1}), respectively, and wide linearity range over the range of 1.8–450 μg L^{-1}. The method has also provided acceptable precisions (%RSD, 0.4–9.4) and satisfactory recoveries over the range of 73 to 109%, with the exception of NS in lake water which was around 50% and PS in river water which was not measured due to the presence of interfering peak. Generally, the obtained results indicated that the developed method could be effectively used as a simple alternative for rapid sample extraction, preconcentration and determination of the target pesticides in water samples and other related matricies.

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Figure captions

Fig. 1 Chemical structures, common names, abbreviation and pK_a of the sulfonylurea and organophosphorus pesticides considered in the study.

Fig. 2 Schematic diagram of the proposed ion-pair extraction procedure.

Fig. 3 Effect of ion-pair concentration. Experimental conditions: volume of TBAHS, 200 µL; volume of acetonitrile, 2 mL; sample pH, 8; salt concentration, 25% NaCl (m/v); sonication time, 20 min; centrifugation rate and time, 4000 rpm and 5 min, respectively.

Fig. 4 Effect of acetonitrile volume. Experimental conditions: concentration of TBAHS, 5 mmol L^{-1}; volume of TBAHS, 100 µL; sample pH, 8; salt concentration, 25% NaCl (m/v); sonication time, 20 min; centrifugation rate and time, 4000 rpm and 5 min, respectively.

Fig. 5 Effect of sample pH. Experimental conditions: concentration of TBAHS, 5 mmol L^{-1}; volume of TBAHS, 100 µL; acetonitrile volume, 1.5 mL; salt concentration, 25% NaCl (*m/v*); sonication time, 20 min; centrifugation rate and time, 4000 rpm and 5 min, respectively.

Fig. 6 Chromatograms (a), (c) and (e) show blanks (unspiked samples) of river water, lake water and ground water samples, respectively. Chromatograms (b), (d) and (f) corrospond to river water, lake water and ground water simple, respectively, spiked with 60 µg L^{-1} for NS, Meth, Fen and Diaz; 40 µg L^{-1} for MSM, RS, PS and Chlor as well as 30 µg L^{-1} for CSE and TSM.

Table 1 Statistics and performance characteristics of the proposed method.

Analyte	Linear range (µg L^{-1})	$S_{y/x}$	Slope (S_b)	Intercept (S_a)	R^2	LOD (µg L^{-1})	LOQ (µg L^{-1})
NS	10.0–450	0.9	0.1 (0.001)	3.0 (0.4)	0.997	3.0	10.0
MSM	3.1–300	1.8	0.4 (0.004)	1.4 (0.7)	0.997	0.9	3.1
RS	5.3–300	1.5	0.3 (0.003)	3.2 (0.5)	0.996	1.6	5.3
PS	3.6–300	3.4	0.4 (0.007)	1.0 (1.1)	0.993	1.1	3.6
Meth	5.5–450	2.4	0.2 (0.003)	3.7 (0.8)	0.993	1.6	5.5
CSE	2.3–225	2.2	0.5 (0.006)	1.3 (0.7)	0.996	0.7	2.3
TSM	1.8–225	2.5	0.7 (0.007)	0.6 (0.8)	0.997	0.5	1.8
Fen	7.1–450	1.2	0.1 (0.001)	3.1 (0.4)	0.996	2.1	7.1
Diaz	8.0–450	0.9	0.1 (0.001)	1.9 (0.3)	0.995	2.4	8.0
Chlor	4.8–300	1.4	0.2 (0.002)	2.1 (0.5)	0.996	1.4	4.8

$S_{y/x}$, S_b and S_a are standard deviation of the residuals, slope and intercept, respectively; R, coefficient of determination

Table 2 Intra- and inter-day precisions of the proposed method (%RSD) for spiked groundwater samples.

Analyte	Intra-day (% RSD, n = 6)			Inter-day (% RSD, n = 15)		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
NS	2.6	3.3	4.0	5.2	6.2	5.4
MSM	1.2	1.4	3.6	9.4	5.8	7.5
RS	2.4	1.1	2.0	8.2	5.6	2.5
PS	4.8	0.4	3.3	7.3	3.7	4.5
Meth	3.5	0.4	3.3	9.7	3.8	4.4
CSE	3.8	0.6	3.1	8.7	7.4	4.3
TSM	4.0	1.2	3.5	6.0	2.4	3.9
Fen	3.2	5.5	3.3	9.2	9.0	4.2
Diaz	3.4	6.6	5.8	4.3	3.5	8.3
Chlor	4.0	2.2	4.9	9.0	4.6	6.8

Level 1: 60 μg L^{-1} for NS, Meth, Fen and Diaz; 40 μg L^{-1} for MSM, RS, PS and Chlor; 30 μg L^{-1} for CSE and TSM

Level 2: 180 μg L^{-1} for NS, Meth, Fen and Diaz; 120 μg L^{-1} for MSM, RS, PS and Chlor; 90 μg L^{-1} for CSE and TSM

Level 3: 360 μg L^{-1} for NS, Meth, Fen and Diaz; 270 μg L^{-1} for MSM, RS, PS and Chlor; 180 μg L^{-1} for CSE and TSM

Table 3 Relative recoveries (%RR) of the proposed method for water samples.

Analyte	Groundwater (%RSD, n = 6)			River water (%RSD, n = 6)			Lake water (%RSD, n = 6)		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
NS	82 (2.6)	92 (3.3)	78 (4.0)	81 (8.3)	77 (2.1)	83 (1.2)	53 (3.3)	50 (2.7)	52 (6.9)
MSM	105 (1.2)	73 (1.4)	82 (3.6)	103 (3.0)	103 (9.4)	99 (0.7)	104 (1.9)	96 (5.4)	96 (5.9)
RS	91 (2.4)	82 (1.1)	85 (2.0)	82 (4.0)	89 (1.1)	91 (0.8)	93 (3.5)	98 (1.1)	99 (1.7)
PS	104 (4.8)	101 (0.4)	101 (3.3)	***	***	***	88 (6.4)	97 (1.6)	100 (0.4)
Meth	93 (3.5)	81 (0.4)	89 (3.3)	105 (2.1)	109 (1.1)	107 (1.2)	101 (4.1)	96 (1.5)	94 (1.0)
CSE	105 (3.8)	77 (0.6)	88 (3.1)	102 (5.2)	102 (0.9)	102 (1.1)	93 (3.6)	90 (1.4)	88 (0.7)
TSM	99 (4.0)	75 (1.2)	88 (3.5)	108 (4.2)	99 (0.8)	100 (1.0)	102 (3.7)	94 (1.2)	92 (0.5)
Fen	93 (3.2)	95 (5.5)	91 (3.3)	106 (3.8)	99 (0.9)	101 (0.9)	96 (4.2)	95 (2.7)	92 (0.8)
Diaz	78 (3.4)	99 (6.6)	100 (5.8)	100 (2.6)	103 (3.4)	104 (3.5)	97 (2.7)	96 (1.8)	91 (1.8)
Chlor	94 (4.0)	83 (2.2)	99 (4.9)	101 (6.4)	100 (1.3)	98 (2.9)	86 (6.4)	89 (5.5)	88 (1.9)

Level 1: 60 μg L^{-1} for NS, Meth, Fen and Diaz; 40 μg L^{-1} for MSM, RS, PS and Chlor; 30 μg L^{-1} for CSE and TSM

Level 2: 180 μg L^{-1} for NS, Meth, Fen and Diaz; 120 μg L^{-1} for MSM, RS, PS and Chlor; 90 μg L^{-1} for CSE and TSM

Level 3: 360 μg L^{-1} for NS, Meth, Fen and Diaz; 270 μg L^{-1} for MSM, RS, PS and Chlor; 180 μg L^{-1} for CSE and TSM

**** not measured due to the presence of interfering peak*

Table 4 Comparison of the proposed method with others reported methods for extraction and determination of pesticides multiresidues.

Method	Analytes	Linear range ($\mu g\ L^{-1}$)	LOD ($\mu g\ L^{-1}$)	%RSD	Extraction time (min)	Sample volume (mL)	Ref.
SPE-HPLC-UV	SUs	15–150	9.4–14.5	–	> 60	1000	14
	SUs	15–150	5.0–8.1	–			
SPE-HPLC-MS^n							
IL-MNPs based SPE-CLC*-DAD	SUs	5–100	1.1–2.9	2.3–4.9	–	50	43
Au-TEOS based SPE Au-NP-IL-silica based SPE–CLC-DAD C_{18} based SPE	SUs	50–1000	2.0–9.0	2.1–4.5	–	10	44
HF-LPME-CLC-DAD	SUs	0.3–40	0.1–1.7	0.9–8.4	60	12	40
CPE-HPLC-DAD	SUs	4–2000	0.8–1.2	0.4–5.9	17	18	45
IPA-LLE-HPLC-DAD	SUs & OPs	2–450	0.9–3.0	0.4–9.7	20	5	This work

**CLC: capillary liquid chromatography*

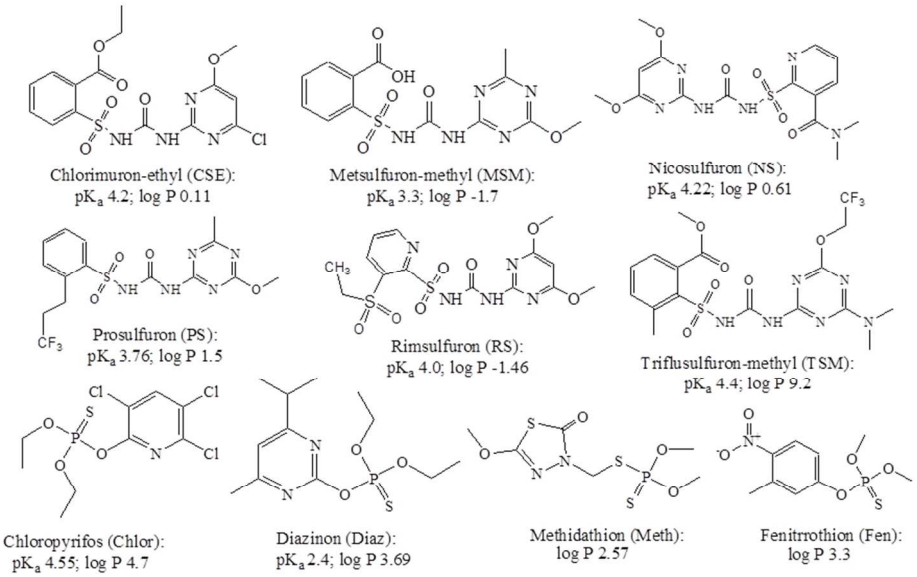

Fig. 1

254x190mm (96 x 96 DPI)

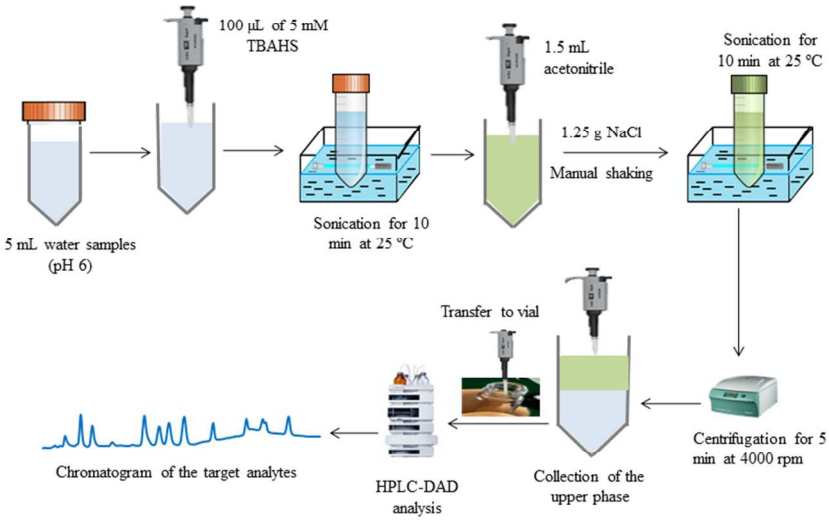

Fig. 2

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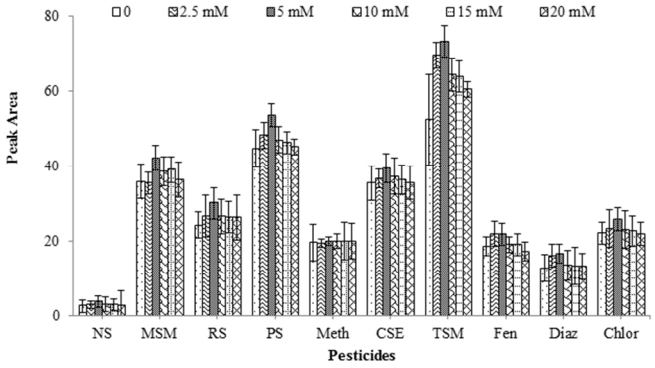

Fig. 3

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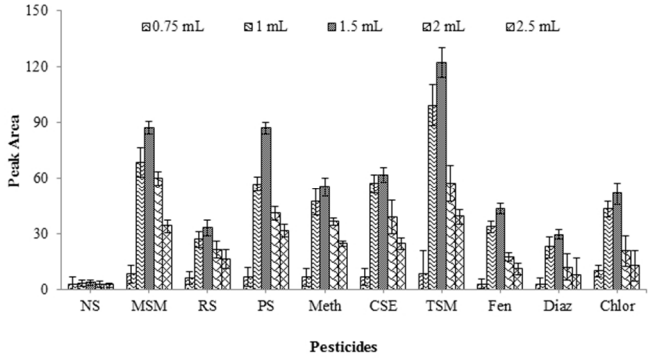

Fig. 4

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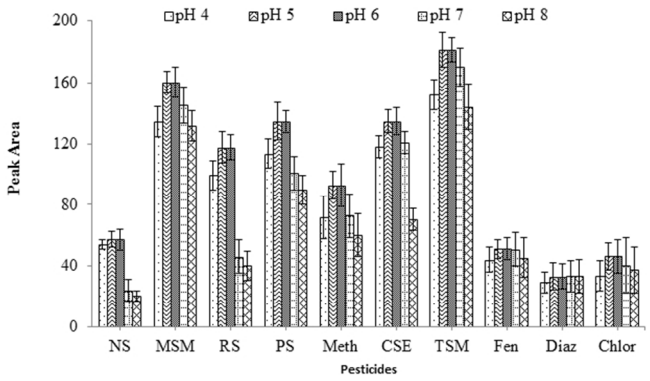

Fig. 5

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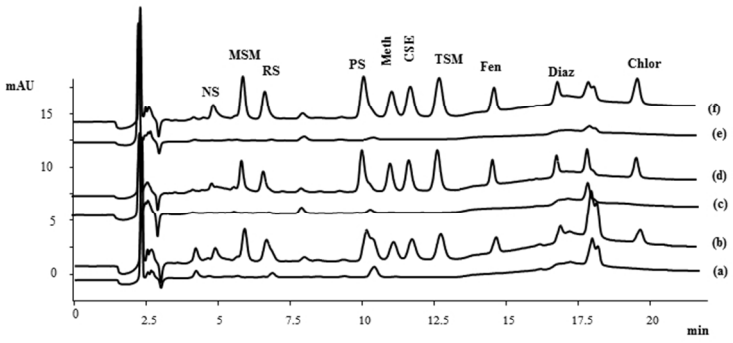

Fig. 6

254x190mm (96 x 96 DPI)