

Development of a new liquid phase microextraction method with hollow fiber HF-SBME for the analysis of the organochlorine compounds in water samples by GC-ECD

Jhon Alexander Fiscal Ladino¹

Sandra Liliana Correa Chacón¹

Sandra Ceballos Loaiza²

Alberto de la Ossa Salcedo²

Gonzalo Taborda Ocampo¹

Cristina Nerin³

Milton Rosero-Moreano^{1*}

¹Universidad de Caldas, Facultad de Ciencias Exactas y Naturales, Depto. Química, Sede Principal Calle 65 No 26-10 A.A 275 Manizales-Colombia

²Dirección Territorial de Salud de Caldas DTSC, Laboratorio de Salud Pública, Área de Análisis Instrumental

³Universidad de Zaragoza, EINA Departamento de Química Analítica Campus Rio Ebro, Calle María Luna 9 50018, Zaragoza-España

*milton.rosero@ucaldas.edu.co

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Abstract

A novel liquid phase microextraction (LPME) method has been developed, by coating with hollow fiber a metallic paper pin. Into of lumen of 30 mm of accurel membrane (300/1200 PP® Membrana GmbH Wuppertal-Germany) was put a 10 mm metallic piece, functionalizing the hollow fiber as a solvent bar and was used for the organochlorine compounds extraction from several aqueous samples. This new technique was coined as hollow fiber solvent bar microextraction HF-SBME, and has taken the advantages of the liquid phase microextraction with membrane such as: to serve as barrier for interferences, has a high enrichment factor, cheap and disposable to avoid carry over for one side and the skills of the stir bar sorption extraction such as: a big surface contact, a big mass transference capability and great facilities to recover the bar for the other side. The optimal conditions for the new microextraction method were: 1-octanol as organic solvent filled into membranes's lumen (mode two phases); 20 min as extraction time; 700 rpm as stirring speed; 30 °C as temperature extraction; 20% NaCl as salting out effect. Under these conditions and after extraction procedure was injected 1 µL of extracted solvent from membrane's lumen with Hamilton 10 µL syringe, and used a gas chromatography equipped with electron capture detector GC-ECD for the analysis of six organochlorine (except carbaryl) pesticide (endrin, dieldrin, 4,4'-DDD, aldrin, lindane) compounds in several aqueous samples as a leachate landfill and drinking water.

Keywords: Organochlorine compounds, liquid phase microextraction, HF-SBME, CG-ECD; pesticide; leachate; drinking water.

1. Introduction

Emerging pollutants such as pharmaceuticals, personal care products (PPCPs) and disinfection byproducts (DBPs) are nowadays detected in rivers, water sources and many other environmental samples. An emerging pollutant corresponds to an unregulated compound which is a candidate for further future regulation, depending on their potential effects on health and the environment. Disinfection byproducts and other organochlorine compounds include a large number of compounds, for example: trihalomethanes (regulated), haloacetic acids, haloacetonitriles, haloketones, insecticides and pesticides. The introduction of these compounds in the environment comes from different sources: *in situ* formation by reaction between natural organic matter and chlorine in water treatment plants^[1,2], wastewater discharges (treated and untreated) in rivers and seas, discharges from crops where land is prepared by using organochlorine pesticides.

The organochlorine compounds are a group of chemical products used mainly for controlling of crop damage caused by insects, these compounds also have other functions, not only in the agricultural part but also are used as products for disinfection, for this reason the use of these compounds as pesticides are so usefulness for agricultural workers for their crop protection, but these compounds are catalogued as toxics, due to may cause health people and animal damage, where the indiscriminate use is generating critical problems of environmental pollution and in the food products by direct aspersion^[3,4,5].

One of the most important steps in any analytical procedure is sample preparation. Most analyzes are carried out on samples containing complex mixtures with very small amounts of the chemicals such as DBPs and pesticides which need to be identified and/or quantified. At the same time, most sample matrices, such as soils or wastewater, are also very complex. Thus, a successful sample preparation method typically has three major objectives: (1) sample matrix simplification

and/or replacement, (2) analyte enrichment, and (3) sample cleanup^[6]. In addition, a continuous search for improved sample preparation procedures is required with the following goals: (1) reduction in the number of steps required for the procedure, (2) adaptability to field sampling, (3) automatization and (4) reduction or total elimination of solvents required for extraction in agreement with current trends in analytical chemistry and taking into account the green chemistry ideology.

The actual conventional methods in the analysis of aqueous and solid samples use techniques as liquid-liquid extraction (LLE), supercritical fluid extraction (SFE) and solid phase extraction (SPE)^[7,8,9]. Nowadays the LLE is a widely used technique, but has some disadvantages, as the large amount of solvent used, the emulsion formation into the phases and spends large time in the cleanup step. The SPE eliminates the disadvantages of LLE, but the presence of the matter in the particles in the samples may cause the tamponade of cartridges and the required sample volume still presents some problems with the SPE applications.

In this way, the liquid phase microextraction (LPME) techniques have gained a lot of interest because they fully comply with the objectives and goals established above^[6]. Actually a new and easy technique is implementing, the liquid phase microextraction LPME has been developed as sample preparation method at small scale (miniaturization) of the conventional LLE^[10]. Small amounts of organic solvent are used to extract the analyte in aqueous matrices. The liquid phase microextraction (LPME) uses low cost devices and very simple arrangement, in this case a hollow fiber is used to protect the solvent, allowing the extraction of all solvent volume immobilized into the membrane pores^[11,12]. This new applied technique with modification of some critical variables allows with success to develop some research works. The LPME provides an efficient preconcentration and sample cleanup step, due to the selectivity and membrane polarity with the target analyte, and the recovered extract may inject directly to GC^[13,14].

The LPME has been used in the last time with some great variety: in two phases mode; in three phases mode, in U-shape form; in vertical shape form; in S-shape form involved a fixed barrel and a free form put into container donor phase. However in all these cases has been mandatory to use the LPME system accompanied with a magnetic stir for improving the mass transference and reduce the extraction time to reach the equilibrium. The aim of this work is to functionalize the hollow fiber as solvent bar allowing the extraction of organochlorine compounds from several aqueous samples with an easy, cheap, fast, robust, efficient, high preconcentrated, high clean up method and with big possibilities to automatize^[15,16,17,18,19,20,21,22].

This research propends to determine the applicability of the liquid phase microextraction with hollow fiber (HF-LPME) by gas chromatography coupled with electron capture detector (ECD) for the determination of six pesticides commonly used by farmers at the coffee cropland in the Caldas State (such as Carbaryl, Endrin, Dieldrin, 4,4'-DDD, Aldrin, and

Lindane see Figure 1) in water samples. Modifying the critical parameters that affect to the extraction of the studied pesticides such as the proper organic solvent selection, stirring speed, extraction temperature and time, salting out effect (NaCl), always searching optimize this variables for reach a good efficiency, selectivity and sensitivity of the procedure.

2. Experimental Procedure

2.1. Reagents and standard solutions

All reagents and standards were purchased from Sigma Aldrich Company through authorized companies in Colombia (Quimirel Ltd. and Outsourcing Ltd.). From the pure standards of five (5) organochlorine compounds such as: Endrin, Dieldrin, 4,4'-DDD, Aldrin and Lindane, and other pesticide such as Carbaryl were prepared stock solutions of 5 mg L⁻¹ of each compound with isooctane, the other work solutions were prepared by dilution from the stock solutions with ultrapure water type I (obtained with Millipore deionized equipped). The standard, stock and working solutions were stored at 4 °C.

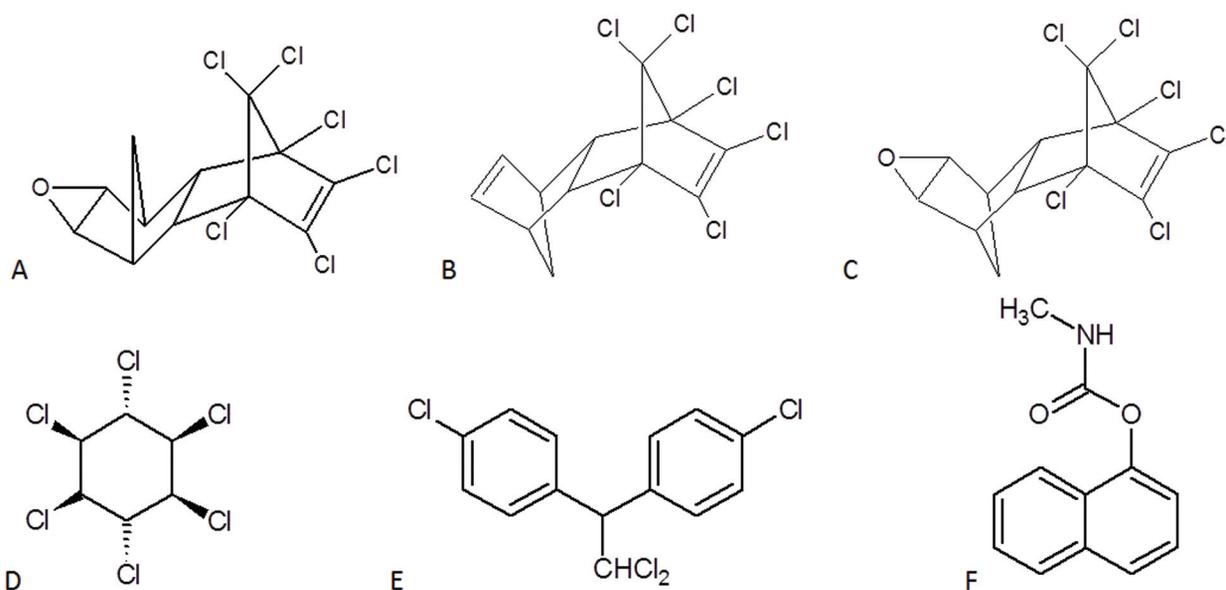


Figure 1. Molecular structures of pesticides most widely used in control of pest in Colombian crops, used as molecular models for the microextraction of organochlorine compounds in water samples. (A) Endrin, (B) Aldrin, (C) Dieldrin, (D) Lindane, (E) 4,4'-DDD and (F) Carbaryl.

2.2. Chromatographic conditions in GC – ECD system

A Thermo Trace 100 Gas Chromatograph equipped with ECD detector was used in this experiment. The detector temperature was set at 300 °C. The Column was an Rtx®-5 30 m × 0.32 mm ID × 0.25 m. The oven temperature ramp was as follows: Initial temperature of 150 °C, then it was increased at 10 °C min⁻¹ to 240 °C, then the temperature was increased at 20 °C min⁻¹ to 290 °C was held for 10 min more, finally was used a 8 min as a post-run analysis. The run time was 35 min. The injector temperature was at 250 °C and was used in splitless injection mode. The injection volume was 1 µL. The carrier gas was helium (99.999% purity) at a flow rate of 1.0 mL min⁻¹; the auxiliary gas was nitrogen (99,999%, 30 mL min⁻¹) supplied by Lynde Gas company.

2.3. Hollow fiber specifications^[6]

The hollow fiber HF was preconditioned under acetone and in an ultrasonic bath for 10 min., and then allowed to air dry. The HF is made of polypropylene. The length of HF was 30 mm, the film thickness was 300 µm, with a pore size of 0.2 µm and internal diameter ID of 1.2 mm. The specific surface area was 17.3 m² g⁻¹. The trade mark reference was Accurel PP 300/1200 acquired from GmbH Polypore Cia, Germany.

2.4. Microextraction conditions^[14]

After applying an $N+(N-1)$ design for the optimization of the critic variables in the microextraction procedure, the best conditions for the operation of the HF-SBME system were obtained (see Figure 2). The conditions were as follows: extraction solvent: 1-octanol; extraction temperature: 30 °C; extraction time: 20 min.; stir speed: 700 rpm. sample volume: 50 mL.; solvent volume: 40 µL; salting out effect: 20% NaCl.

2.5. Step by step microextraction procedure^[14]

The newly developed method involved the following steps (see Figure 3) : 1). Preconditioning the hollow fiber under acetone in an ultrasonic bath for 10 min.; 2). Functionalizing the hollow fiber as a solvent

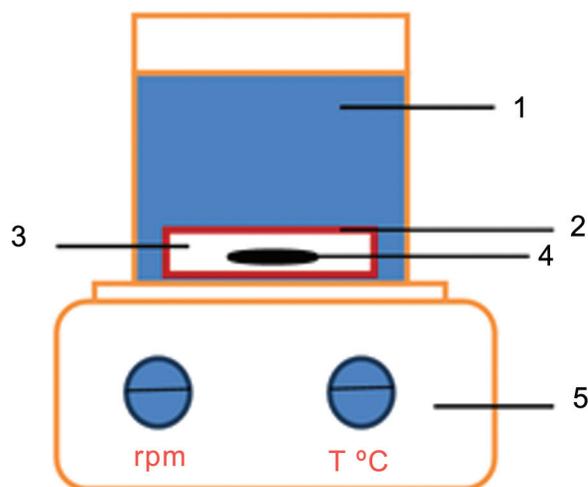


Figure 2. Arrangement of HF-SBME system: 1. Donor phase; 2. Hollow fiber sealed in both edges; 3. Acceptor phase; 4. Metallic paper pin; 5. Stir/hot plate.

bar by coating the 10 mm metallic paper pin with 30 mm of hollow fiber; 3). Filling the hollow fiber with 40 µL of solvent (1-octanol) using a 10-µL Hamilton syringe; 4). Sealing both edges of the hollow fiber by mechanical pressure with lab tweezers; 5). Putting the functionalized HF-SBME into spiked water sample; 6). Operating the HF-SBME into spiked water sample; 6). Operating the HF-SBME system under the optimized conditions (700 rpm stirring speed, 30 °C extraction temperature, 20% NaCl salting out effect); 7). Obtaining the HF-SBME after 20 min of extraction; 8). Cutting one of the hollow fiber's edge and recovering the concentrated extract using a 10-µL Hamilton syringe; and 9). Injecting 1 µL of extract into a gas chromatograph equipped with an electron capture detector GC-ECD. After each trial, the hollow fiber was discarded to avoid interferences and carry over effects.

2.6. Spiked and real samples

The 50 mL ultrapure water type I (milliQ system) was spiked with 5 µg mL⁻¹ of the six organochlorine compounds: lindane, endrin, dieldrin, aldrin, DDD and carbaryl, which are the most common pesticide used in Colombian crops. This spiked water sample was used for the optimization procedure of the HF-SBME microextraction system.

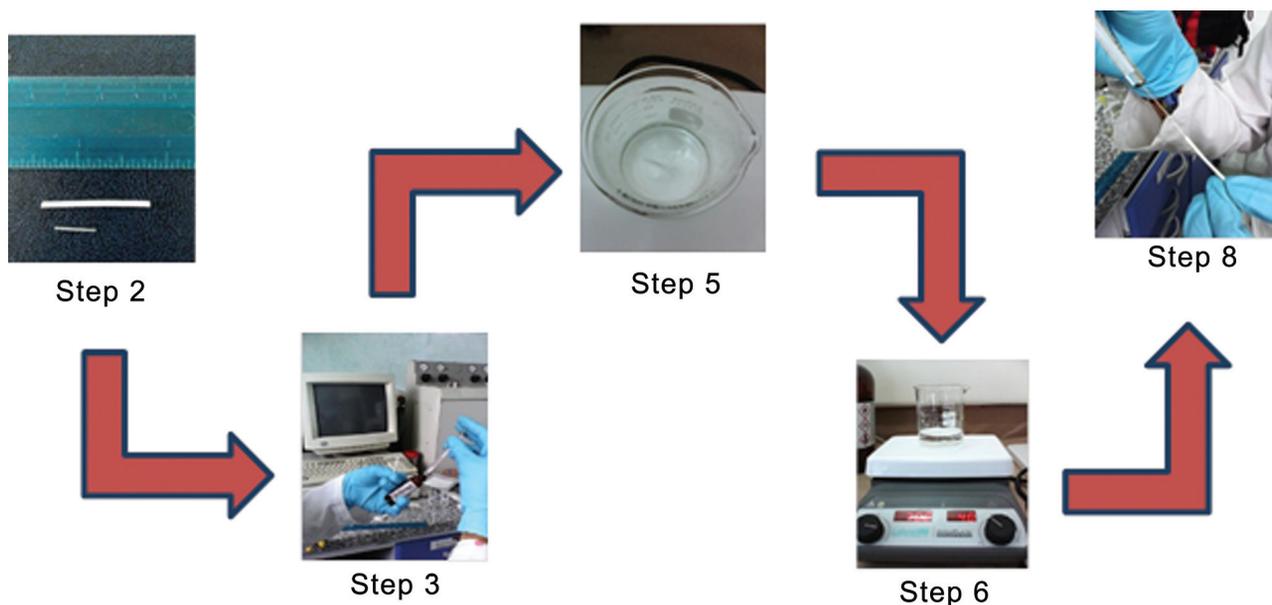


Figure 3. Step by step of microextraction procedure with HF-SBME system.

The optimized method was then assayed using real samples: drinking water and leachate landfill. These samples were collected the first one from the Health Public Laboratory tap water by adjusting de flow to 500 mL min^{-1} and the second one sample, the leachate was collected from the landfill of the Manizales city located at 10 min by car in road to Neira's municipality. These samples were collected and immediately analyzed.

3. Results and Discussion

3.1. Optimization procedure

For the implementation of this new and easy method using a hollow fiber as a solvent bar for the microextraction of organochlorine compounds for the determination of Carbaryl, Endrin, Dieldrin, 4,4'-DDD, Aldrin, and Lindane in aqueous samples, several critical parameters were taken into account to obtain the optimal yield. The parameters included the extraction temperature ($N1$), the extraction time ($N2$) and the effect of salting out ($N3$). An " $N + (N-1)$ rule" of experimental design was used to evaluate these effects on the microextraction procedure^[6,15].

Finally, twenty-one microextraction assays (seven by triplicate) were conducted. In this case, N critical variables were taken into account ($N1-N3$) with three levels each, three Ns were added, subtracting one from each of them except the first one: [$N1 + (N2 - 1) + (N3 - 1)$]. So in total $3 + (3 - 1) + (3 - 1) = 7$ assays were needed.

3.1.1. Effect of stirring speed of sample

Due to the hollow fiber was functionalized as solvent bar and that in this case the real stirring speed transferred from the stir/hot plate to HF-SB is impossible to advertise, and for to avoid significant losses or formation air bubbles that occupied the pores of HF-SB preventing the exchange between donor and acceptor phases in a good way and limiting the extraction, we resolve to fix this condition at 700 rpm.

3.1.2. Effect of extraction temperature

The temperature is an essential parameter in the extraction procedure that influences the transference rate and the partition coefficients of the analytes. The temperature was changed to 25°C , 30°C and 40°C during the extraction of spiked water sample with $5 \mu\text{g mL}^{-1}$ of the pesticides standard solution at a stirring speed

of 700 rpm for 20 min, was achieved demonstrate that increasing the temperature increases analytes extraction but some compounds do in a larger quantities as shown in Figure 4, Due to increasing of temperature in general can improve the partition coefficient of some analytes and there were no significant increases at 40 °C in the extraction inclusively was a worst trial, we choose as an optimal temperature to apply the 30 °C.

3.1.3. Effect of extraction time

The evaluated time points were: 10 min, 20 min and 40 min. The optimal extraction time in the extraction of spiked water sample with 5 $\mu\text{g mL}^{-1}$ of pesticide compounds at 30°C as extraction temperature and 700 rpm as stirring speed, the increasing of analyte's extraction is achievable demonstrate in the Figure 5, until 20 min of extraction period, then the change is not enough noticeable. However, the prolonged extraction time not represent substantial improvements but may cause loss of solvent in the sample solution in special in this new kind of arrangement when the HF was functionalized as solvent bar, in this way was established 20 min as optimal extraction time.

3.1.4. Effect of salting out addition

In general, the extraction of organochlorine compounds with this new method gives good results. Six tested pesticides (Carbaryl, Endrin, Dieldrin, 4,4'-DDD, Aldrin, and Lindane), were extracted from a water sample spiked with 5 $\mu\text{g mL}^{-1}$ under the following conditions: extraction temperature at 30 °C, stirring speed of 700 rpm, 20 min of extraction and 20% NaCl for salting out (see Figure 6).

To improve the target compounds extraction, NaCl is added to increase the ionic strenght of the sample solution, to increase the solubility of the target analyte in the organic phase and improve the analyte recovery. For this microextraction method, in agreement with other authors, the spiked water sample with 5 $\mu\text{g mL}^{-1}$ was treated with different salt concentrations of 5%, 10% and 20% NaCl, as shown in the Figure 6.

The increasing salting out addition increased the target pesticides extraction, higher salt concentrations were not necessary because based on our experience and the

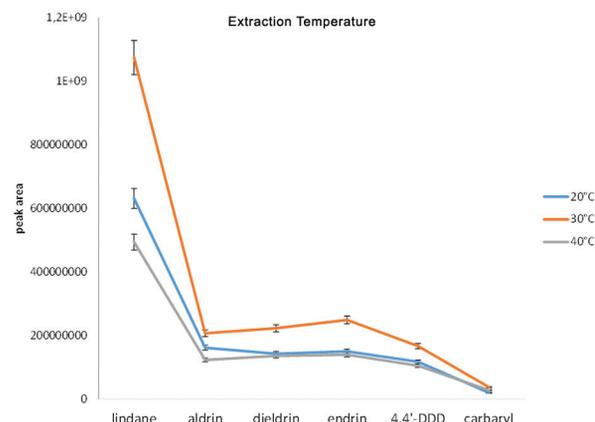


Figure 4. Effect of extraction temperature in the microextraction with HF-SBME system.

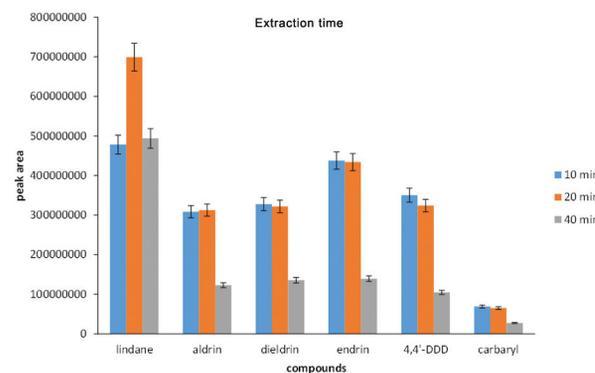


Figure 5. Effect of extraction time in microextraction with HF-SBME system.

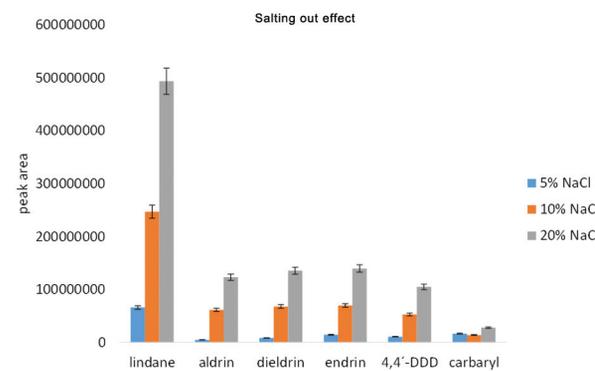


Figure 6. Effect of salting out addition in microextraction with HF-SBME system.

other authors(6); the excess use of ionic strength may affect the analytes diffusion into the organic phase, for this reason was chosen 20% NaCl as salting out concentration for all the future trials in special with the real samples.

3.2. Analysis of real samples

Under the optimized conditions, two water samples were tested: drinking water and leachate landfill. The chosen conditions were as follows: extraction temperature 30 °C; extraction time 20 min.; stirring speed 700 rpm and salting out addition 20% NaCl.

3.2.1. Analytical characteristic of the developed method

For the examination of analytical characteristics of the developed method, a routine analysis was established over six days by assaying five different type of experimental solutions in duplicate: blanks (1-octanol), standard solutions at low concentrations ($0.700 \mu\text{g mL}^{-1}$), standard solutions at high concentrations ($6.0 \mu\text{g mL}^{-1}$), tap water and spiked drinking tap water sample ($+ 5 \mu\text{g mL}^{-1}$).

The analytical characteristic are shown in the Table 1. The LoD was calculated according to the IUPAC rules. In general the method exhibits good performance parameters, with exception for carbaryl due its low solubility in isooctane solvent because was prepared a mix solution of all of them. Although the injection is manual and taking into account the entire procedure (see Figure 3), the relative standard

deviations with an exception for carbaryl were below 10%. The precision could be further improved by automatization or using the internal standard calibration mode.

As shown in the Figure 7, the calibration curves of organochlorine compounds were determined in the same way as the real and spiked samples, with the microextraction by HF-SBME system, in this case the calculations for the sample was directly through the extrapolation with linear equation of calibration curves shown in the Figure 7.

Based on the overlapping chromatograms (see Figure 8), this new HF-SBME system extracted, purified and concentrated all six organochlorine compounds from the spiked water sample.

The results of the recovery test were determined using the following equation: $\%R = [(C_s - C_{ns})/C_s] \times 100$

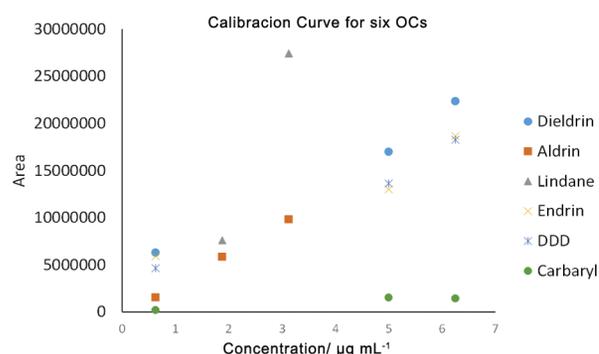


Figure 7. Calibration curves for the six target organochlorine compounds obtained with application of the HF-SBME system under the optimized conditions.

Table 1. Analytical characteristics of the new develop method HF-SBME system.

| Compound | LOD [ng mL^{-1}] | Lineal range [$\mu\text{g mL}^{-1}$] | R^2 | RSD [%] n = 6 | Recovery [%] | Enrichment factor |
|----------|-----------------------------|--|-------|---------------|--------------|-------------------|
| LINDANE | 27,0 | 0,625-6,25 | 0,910 | 10,3 | 89±9 | 200 |
| DIELDRIN | 29,0 | 0,625-6,25 | 0,995 | 9,7 | 73±7 | 150 |
| ALDRIN | 37,0 | 0,625-6,25 | 0,999 | 8,7 | 87±3 | 190 |
| ENDRIN | 47,0 | 0,625-6,25 | 0,942 | 10,8 | 95±2 | 250 |
| 4,4'-DDD | 67,0 | 0,625-6,25 | 0,984 | 10,4 | 83±3 | 180 |
| CARBARYL | 100,8 | 0,625-6,25 | 0,928 | 15,9 | 54±8 | 100 |

where C_s is the concentration of spiked sample and C_{ns} is the concentration of non spiked or natural sample. As shown in the Figure 8, all six of the organochlorine compounds spiked in tap drinking water with exception of carbaryl showed a good signal.

3.2.2. Laboratory tap and drinking water

As shown in the Figure 9, at low concentrations (ng mL^{-1}), the presence of at least three pesticides: Diendrin, Aldrin and 4,4'-DDD were detected in the laboratory drinking tap water, which means there is an

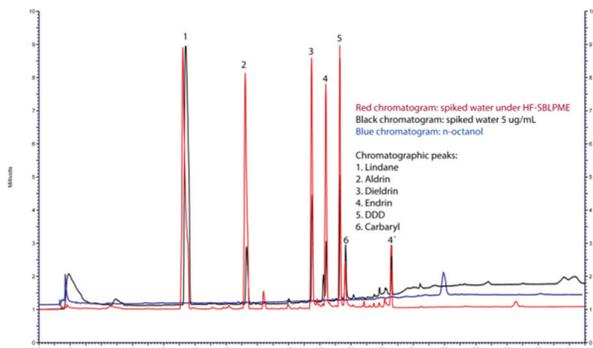


Figure 8. Overlapping chromatograms of the recovery study under the optimized conditions.

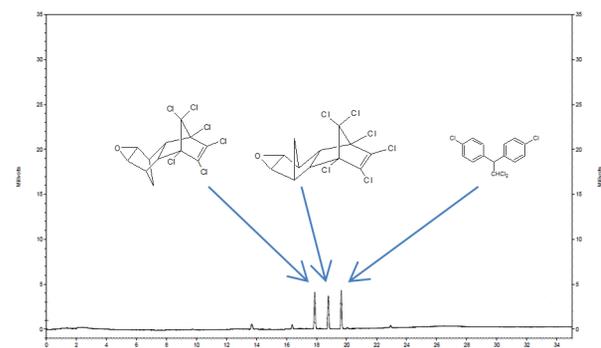


Figure 9. Tap drinking water chromatogram after microextraction procedure with HF-SBME system.

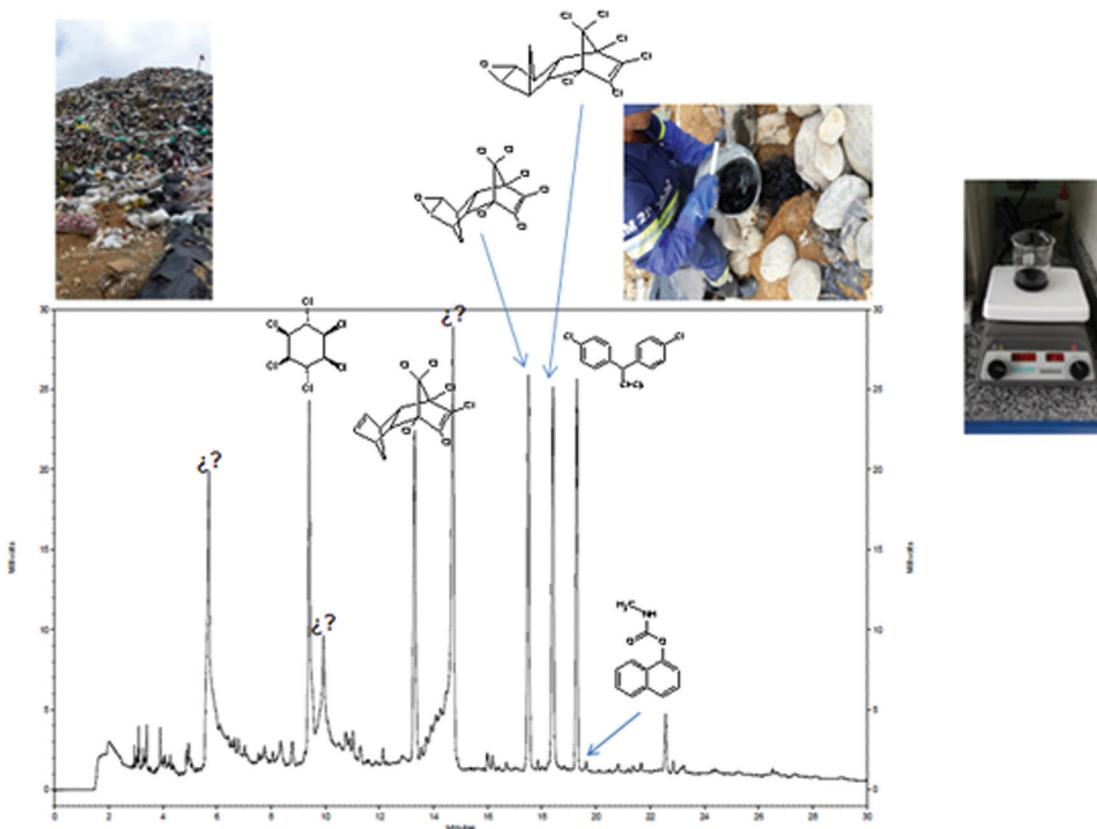


Figure 10. Leachate landfill sample under optimized microextraction conditions with HF-SBME system.

influence of the cropland beside to the water treatment plant because these compounds are passing through the treatment without control and represent a risk for the population served.

3.2.3. Leachate landfill water

As shown in the Figure 10, at high concentrations ($\mu\text{g mL}^{-1}$) the presence of the six all target organochlorine pesticides compounds were detected. This fact is no new and even less surprising due to the high organic matter content in this kind of matrix and the actual disposal system in all the Colombian cities of solid waste is far to avoid the presence of this compounds because there isn't a really control and classification in the source of the garbage before to the disposal in the landfill that means no just organochlorine and organophosphate, PCBs, POPs (persistent organic pollutant) will continue arrive to this sites until an effective and proper checks and true classification are taken.

In the other side, despite the leachate landfill is a recalcitrant and heavy matrix with high organic content and other persistent pollutants (as heavy metals, nitrite compounds, fat, etc.), with this microextraction technique by using the new HF-SBME system coupled to GC-ECD system for separation and detection was easy, economic, robust, reproducible, reliable and fast for the organochlorine compounds analysis and may constitute in a real alternative for other wasteful and high cost sample preparation methods for this kind of sample.

4. Conclusions

Has been developed a liquid phase microextraction method in two phases, that involves the coating of a small metallic piece (paper pin 10 mm) with polypropylene (PP) hollow fiber (30 mm) that had worked as a solvent

bar and the filling of lumen's fiber with organic solvent, this system we have coined the name "HF-SBME" from "hollow fiber-solvent bar microextraction"⁴. From this, the aim of this work is the microextraction of organochlorine compounds from water samples, cataloged as "emerging contaminants"^[1,6] with a proven high risk to human health.

From the preliminary results is possible to recognize the potential use in the sample preparation field for this new developed microextraction method because fully comply with the trends and proposal of the sample preparation techniques: low quantity of solvent organic was used (40 μL); the extraction, cleanup and concentration were done in one step; and the HF-SBME system has a big possibilities to the automatization.

In the future we have the idea to use liquid ionics as extraction solvent filling the hollow fiber's lumen for tuning even more the polarity of target molecules with solvent for increasing the selectivity of technique.

Finally the new sample preconcentration method (HF-SBME) is easy and economic (each 30 mm HF costs USD 0,02), thus for each trial is possible to use a new piece of HF and to avoid the carry over effect, has a high enrichment factor allowing low detection limits and as shown from chromatograms of difficult matrices does an impeccable cleaning up step avoiding the matrix interferences.

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