Brazilian Journal of Analytical Chemistry
Pre-publication – Accepted after peer review
doi: 10.30744/brjac.2179-3425.TN-10-2024

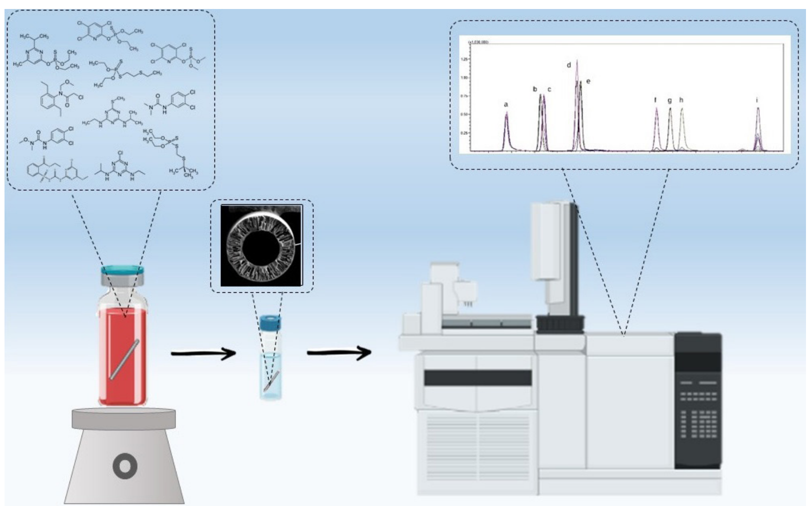
TECHNICAL NOTE

Optimization of the Hollow Fiber Microextraction conditions for the Determination of Pesticides in Whole Blood by GC-MS

Carolina Schmeiske[1,2], Dayse Fernanda de Souza[1,2], Bruno José Gonçalves da Silva[1*]

[1]Departamento de Química, Universidade Federal do Paraná, 81530-000 Curitiba, PR, Brazil

[2]Laboratório de Toxicologia Forense, Polícia Científica do Paraná, 81020-430 Curitiba, PR, Brazil



In forensic toxicology, the sample preparation procedure has been a crucial step in the analytical method development. The main objectives of the sample preparation include the extraction and pre-concentration of the analytes and removal of interfering species, such as endogenous compounds in the biological samples. In recent years, microextraction techniques have been generating interest due to their advantages, especially because the solvent volumes employed during the extraction are greatly reduced, contributing to the principles of green chemistry. In this work, a method based on hollow fiber microextraction (HFμE) and gas chromatography coupled to mass spectrometry (GC-MS) was evaluated for determination of pesticides (chlorpyrifos, chlorpyrifos methyl, diazinon, disulfoton, terbufos alachlor, ametryn, and atrazine) in postmortem whole blood samples. The extraction procedure was performed using polypropylene fibers as device and 1-octanol as extraction solvent. The fiber was immersed under magnetic stirring in the donor phase, which consisted of 500 microliters of whole blood and 7.5 milliliters of buffer solution. After the extraction time, the fiber was transferred to a vial containing an organic solvent and the analytes were desorbed by stirring. The extraction procedure was optimized. In the desorption step, desorption solvent, agitation mode, and time were evaluated. Multivariate optimization of pH, agitation speed, and salt addition were also performed, followed by univariate optimization of extraction time. After optimization, the extraction conditions that showed the best response were: pH adjustment to value 6, stirring at 600 rpm for 40 minutes without salt addition. For the desorption step, 1 minute of vortex agitation using ethyl acetate as the desorption solvent was defined as the optimal desorption condition. Under these conditions, the pesticides were successfully extracted from postmortem whole blood samples.

Cite: Schmeiske, C.; de Souza, D. F.; da Silva, B. J. G. Optimization of the Hollow Fiber Microextraction conditions for the Determination of Pesticides in Whole Blood by GC-MS. *Braz. J. Anal. Chem.* (Forthcoming). http://dx.doi.org/10.30744/brjac.2179-3425.TN-10-2024.

Submitted February 23, 2024; Resubmitted April 18, 2024, 2nd time Resubmitted June 6, 2024, Accepted June 17, 2024; Available online July 2024.

Keywords: microextraction, hollow fiber, pesticides, postmortem blood

INTRODUCTION

Forensic toxicology is defined as the application of toxicology in a legal context. One of the fields of forensic toxicology is postmortem toxicology, or death investigation toxicology.[1] The aim of postmortem toxicology has been to evaluate if a certain compound was the cause of death, if it somehow contributed to it, or if it caused some subject incapacitation.[2,3]

Among the groups of substances of forensic interest, pesticides stand out in Brazil as the country is one of the largest consumers of these substances in the world.[4,5] A study of the data of deaths by intoxication that occurred in Brazil in the period between 2010 and 2015 showed that pesticides were the main toxic agent envolved.[6]

Several matrices can be used in postmortem toxicological analysis. Between them, blood is the selected matrix for quantitative analysis and correlating the concentrations found with pharmacological and toxic effects.[7,8] Postmortem samples are, usually, more complex than samples collected from living subjects due to processes of autolysis and putrefaction. In postmortem blood, separation of red blood cells from serum is usually not possible, so whole blood must be used. Whole blood samples present a great variety of endogenous compounds, such as fatty acids and cholesterol.[2,9,10] In this context, sample preparation has been a very important step in toxicological analysis to concentrate analytes and eliminate matrix interferents before injection in the chromatographic system.[7,11]

Liquid-liquid extraction (LLE) is a simple procedure still widely used in forensic toxicology. LLE is based on the partitioning of the analytes between the sample and several immiscible organic solvents. Although it is a simple and generally efficient technique, it has disadvantages like the great consumption of toxic solvents.[12,13] Motivated by the green chemistry principles, research in miniaturized techniques of extraction that use a minimal amount of solvent, or no solvent at all, have been developed.[12,14]

The miniaturization of classic LLE led to liquid-phase microextraction techniques.[15] Several microextraction techniques have been applied to the extraction of pesticides from biological samples, as can be seen in Table I.

Table I. Application of microextraction techniques to the extraction of pesticides from biological matrices

Extraction method	Matrix	Analytes	Instrumental	Ref.
VA-DLLME	Blood/stomach content/liver	Dichlorvos	GC-MS	16
	Urine	Triazol herbicides	GC-MS	17
DLLME	Plasma/serum	Organochlorine pesticides	GC-MS/MS	18
	Blood	Organophosphate pesticides	HPLC-UV	19
	Urine	20 pesticides of various classes	LC-MS/MS	20
	Urine	Malathion	HPLC-UV	21
DLLME-SFO	Blood/urine	Pyrethroid insecticides	GC-MS	22
	Plasma/urine	Organophosphate pesticides / Pyrethroid insetiticides	GC-MS	23

(continued on the next page)

Table I. Application of microextraction techniques to the extraction of pesticides from biological matrices (continued)

Extraction method	Matrix	Analytes	Instrumental	Ref.
DI-SPME	Serum	Carbamates / organophospate pesticides	TD-ESI/MS/MS	24
HF-SBME	Plasma	Organophosphate / Organochlorine pesticides	GC-FID	25
SDME	Urine	Tebuconazole, pendimethalin, DDT, DDE	HPLC-UV	26
DSPE-DLLME	Urine	Chlorpyrifos	HPLC-UV	27

VA: vortex-assisted; DLLME: dispersive liquid-liquid microextraction; DI-SPME: direct immersion solid phase microextraction; HF-SBME: hollow-fiber solvent bar microextraction; SFO: solidification of floating organic droplet; SDME: single drop microextraction; DSPE: dispersive solid phase extraction; GC: gas chromatography; MS: mass spectrometry; TD-ESI: thermal desorption-electrospray ionization; FID: flame ionization detector; HPLC: high performance liquid chromatography; UV: ultraviolet detector.

Between microextraction techniques, several methods are based in the use of hollow fibers. The use of hollow fibers began when Pedersen-Bjergaard and Rasmussen introduced them as a support for the organic solvent, creating the *hollow fiber liquid-phase microextraction* (HF-LPME).[28] In this technique, the pores of a hollow hydrophobic membrane are impregnated with the organic solvent, while its interior (called lumen) is filled with the acceptor phase. This acceptor phase may be the same organic solvent used for pore impregnation (two-phase HF-LPME) or an aqueous phase (three-phase HF-LPME). The fiber is then immersed in a vial containing the sample (donor phase) and the analytes are extracted from the donor phase, through the immobilized solvent in the pores, to the acceptor phase, which is subsequently collected.[29,30]

As the fiber acts as a physical barrier between the sample and acceptor phase, preventing macromolecules from reaching the lumen, HF-LPME is the most widely used liquid-phase microextraction technique for the extraction of complex biological fluids. But the technique still has some disadvantages, such as the need of microsyringes to fill the lumen and recover the extract, which limits the number of samples that can be processed simultaneously and requires operator skills. Still, the contact area between the extraction solvent and the sample is limited, since the fiber remains fixed during the process.[31,32]

In order to simplify the extraction process, eliminating manual operations with the use of microsyringes, Ide and Nogueira developed the *hollow fiber microextraction* (HFμE). In HFμE, a hollow fiber segment is impregnated with the extraction solvent, and then immersed in the donor phase under agitation. At the end of extraction time, the fiber is transferred to a vial containing an organic solvent, which is submitted to agitation to perform analytes desorption.[33,34] This technique has been applied to the determination of polycyclic aromatic hydrocarbons and organochlorine pesticides in matrices such as surface water, residual water, soil, tea, fish liver and tomato.

Several factors can be adjusted to optimize the extraction procedure, including the membrane characteristics, extraction solvent, extraction time, pH, temperature, agitation, salt addition.[30,35-37] Extraction time must, ideally, be as short as possible. Extraction recovery increases with time until equilibrium is reached. During method optimization, equilibrium time can be established through Recovery x Time curves.[30,37]

Donor phase pH must be adjusted in a way that, according to pKa, acidic or basic compounds remain non-ionized, increasing their solubility in the organic solvent.[35,37,38] Temperature and agitation of the system can both decrease extraction time as they increase diffusion coefficients and decrease the viscosity. Agitation is commonly performed via magnetic stirring or ultrasonic bath. However, increasing the temperature is avoided since it can cause bubble formation and solvent loss due to evaporation.[30,35-37] Salt addition can

increase extraction efficiency, specially of polar compounds, due to salting out effect, that decreases the solubility of the compounds in the aqueous phase. On the other hand, interaction of the analytes with the ions, as well as increase of the matrix viscosity, can reduce analytes diffusion.[35-37]

In this work, HFμE technique was evaluated and optimized for extraction of eight pesticides (chlorpyrifos, chlorpyrifos methyl, diazinon, disulfoton, terbufos, alachlor, ametryn, atrazine) in postmortem whole blood samples and determination by GC-MS.

MATERIALS AND METHODS

Analytical standards

Analytical standards of diazinon (98,4%), chlorpyrifos (98,4%), ametryn (95,8%), chlorpyrifos methyl (99,8%), atrazine (98,1%), disulfoton (94%), terbufos (98,6%), alachlor (99,5%), and the internal standard etrimfos (62%) were provided by the Laboratory of Forensic Toxicology of Scientific Police of Paraná. Stock solutions were prepared by dissolving the compounds in methanol (Dinâmica) at 1 mg mL^{-1}. Working solutions of the analytes at 100 μg mL^{-1} were prepared by further dilution of the stock solutions right before analysis.

Reagents and chemicals

1-octanol (Sigma-Aldrich) was used as extraction solvent and ethyl acetate (Mallinckrodt), acetone (J.T.Baker) and hexane (Panreac) were tested as desorption solvents. The influence of salt addition in the extraction was evaluated using sodium sulfate (Synth). Phosphate buffer pH 2 and 6 were prepared with phosphoric acid, potassium phosphate monobasic (both Sigma-Aldrich) and potassium phosphate dibasic (Merck). Acetate buffer pH 4 was prepared with acetic acid and sodium hydroxide (both Merck).

Instrumentation and chromatographic conditions

Gas chromatography assays were performed at GCMS2010 Plus coupled to mass spectrometer TQ 8040 (Shimadzu). Data obtained were treated with GCMS Solution software. Separation was carried out at SH-Rtx-5MS 30.0 m x 0.25 mm x 0.25 μm column (Shimadzu). Helium was used as carrier gas at a flow of 1.0 mL min^{-1}. Oven temperature was set at 75 ºC and maintained for 3 min, followed by an increase of 20 ºC min^{-1} until 205 ºC, then 30 ºC min^{-1} until 250 ºC, which was maintained for 4 min. Injection was performed at splitless mode (injection volume: 1 μL) and injector temperature was set at 300 ºC. Mass spectra were obtained at SIM mode. Transfer line and ionization source temperatures were set at 275 ºC and 250 ºC, respectively.

Whole blood samples

The postmortem whole blood samples used were collected in necropsies performed by the Scientific Police of Paraná. These samples were previously analyzed by the Laboratory of Forensic Toxicology of the Scientific Police of Paraná and proved to be free of the analytes subjects of this study. Samples were stored at -20 ºC and brought at room temperature before being handled.

Extraction procedure

For hollow fiber microextraction, polipropylene fibers with 600 μm i.d., 200 μm wall thickness and 0.2 μm pore diameter were used (Q3/2 Accurel, Membrana). Fibers were cut into 1 cm segments and cleaned in acetone under ultrasonic agitation (Easy Elmassonic, Elma), followed by air drying. The fibers were used only for one extraction procedure and then discarded, due to their low cost. For pores impregnation, the fiber segments were dipped at 1-octanol for 20 seconds, then transferred for a 10 mL vial containing the donor phase. The donor phase was composed of whole blood (500 μL), containing the internal standard (etrimfos), and buffer solution (7.5 mL). The system was maintained under magnetic stirring (KMO2 basic, Ika-Werke). After extraction time, the fiber was transferred to an insert vial containing 100 μL of the desorption solvent.

Optimization of the extraction procedure

For the optimization assays, blank whole blood samples spiked with the analytes at 1.0 µg mL^{-1} and internal standard at 0.5 µg mL^{-1} were used. Optimization of the desorption step was performed following a 2^2 factorial design (Table II). Agitation mode (vortex and ultrasound) and time (1 and 5 minutes) were optimized.

Table II. Factors and levels of desorption optimization

Factor	Level -1	Level +1
Agitation mode	Vortex	Ultrasound
Time (minutes)	1	5

Multivariate optimization of the parameters pH, agitation speed and salt addition were performed following a Box-Behnken design. Factors and levels studied are described in Table III. For pH adjustment, phosphate buffer (pH 2 and 6) and acetate buffer (pH 4) were used. Salt addition was performed with sodium sulfate. At these assays, extraction time was fixed as 30 minutes.

Table III. Factors and levels of the Box-Behnken design for optimization of extraction

Factor	Level -1	Central Point (0)	Level +1
pH	2 (phosphate buffer)	4 (phosphate buffer)	6 (acetate buffer)
Agitation speed (rpm)	600	800	1000
Salt addition (% m/v)	0	2.5	5

After optimization of these parameters, time extraction was optimized by univariate mode (10, 20, 30, 40, 50 and 60 minutes).

RESULTS AND DISCUSSION

Gas chromatography analysis

For GC analysis, etrimfos was used as internal standard. Etrimfos was selected since it has similar properties to the analytes and is banned in Brazil. The chromatogram obtained after the injection of a mix containing the analytes and IS at 1 µg mL^{-1} under the defined conditions can be seen in Figure 1. Information about the retention times obtained for all analytes and internal standard, quantification and confirmation ions selected, as well as some physicochemical properties of the compounds is demonstrated in Table IV.

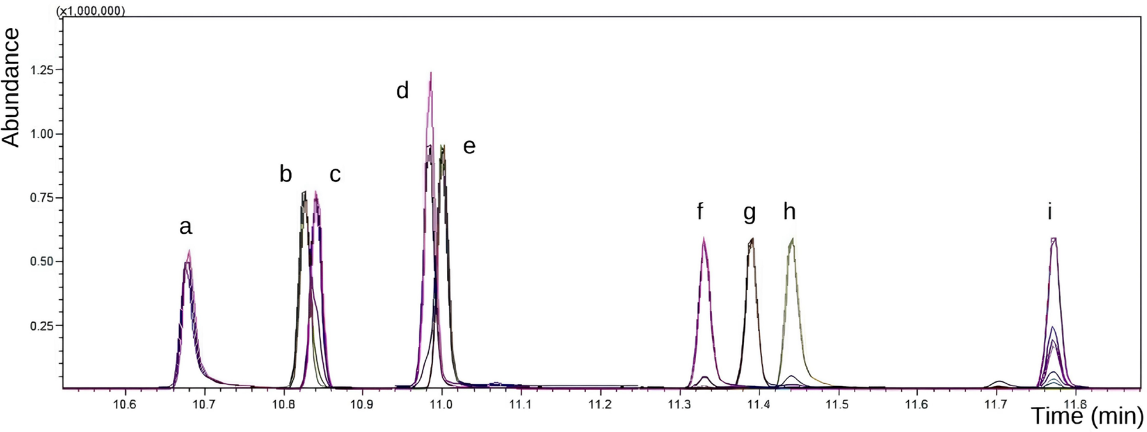


Figure 1. GC-MS chromatogram obtained at SIM mode of a mix containing the analytes and IS at 1 µg mL^{-1}. a: Atrazine; b: Terbufos; c: Diazinon; d: Disulfoton, e: Etrimfos, f: Chlorpyrifos methyl, g: Alachlor, h: Ametryn, i: Chlorpyrifos.

Table IV. Identification parameters and physicochemical properties of the analytes

Compound	Molecular formula	RT (min)	Quantitation ion	Confirmation ions	Molecular weight (g mol^{-1})	pKa	LogP[a]
Atrazine	$C_9H_{11}Cl_3NO_3PS$	10.670	200	215-173	350.6	ND[b]	4.7
Terbufos	$C_7H_7Cl_3NO_3PS$	10.805	231	103-125	322.5	ND[a]	4
Diazinon	$C_{12}H_{21}N_2O_3PS$	10.825	179	137-152	304.35	2,6[b]	3.69
Disulfoton	$C_8H_{19}O_2PS_3$	10.970	88	89-142	274.4	ND[a]	3.95
Chlorpyrifos methyl	$C_9H_{21}O_2PS_3$	11.310	286	288	288.4	ND[a]	4.51
Alachlor	$C_{14}H_{20}ClNO_2$	11.370	160	188-146	269.77	0,62[a]	3.09
Ametryn	$C_9H_{17}N_5S$	11.430	212	227-170	227.33	4,1[b]	2.63
Chlorpyrifos	$C_8H_{14}ClN_5$	11.750	197	199-314	215.68	1,6[a]	2.7
Etrimfos (IS)	$C_{10}H_{17}N_2O_4PS$	10.980	181	292-168	292.29	ND[a]	2.94

References: a[39]; b[40]

As it is possible to see in Table IV, some of the compounds presented very similar retention times, and co-elution was observed between terbufos and diazinon, and between disulfoton and etrimfos. This problem was resolved by selection of monitoring ions for these analytes that weren't present at the mass spectrum of the co-eluted substance.

Selectivity of the chromatographic method was assessed in terms of matrix interferences and interferences from other commonly encountered analytes. To the matrix interferences assay, 10 blank matrix samples from different sources were analyzed by the developed method. A *m/z* ion 227 was detected at ametryn retention time. This ion is the most abundant ion in ametryn mass spectrum, therefore would be chosen as the quantitation ion for this compound. After this study, however, *m/z* 227 was replaced by 212, the second most abundant ion in ametryn mass spectrum. No interference was detected when monitoring the ion 212.

The second selectivity study was performed by injection of a mix containing common medications and drugs of abuse that could be present in real samples (Table V). GC analysis was performed in *full scan* mode and only imipramine and cocaine were detected. Both showed, however, longer retention times than the last eluted analyte. Besides direct injection, this interferences mix was added to a blank matrix at 0.5 mg mL^{-1} concentration, and submitted to the extraction procedure before GC analysis. In this assay, it was possible to verify that the two compounds previously detected (imipramine and cocaine) were not extracted by the developed extraction method.

Table V. Retention time of the analytes and possible interferents studied

Analyte	Retention time (min)
Atrazine	**10.670**
Terbufos	**10.805**
Diazinon	**10.825**
Disulfoton	**10.970**
Etrimfos	**10.980**
Chlorpyrifos methyl	**11.310**
Alachlor	**11.370**
Ametryn	**11.430**
Chlorpyrifos	**11.750**
Etrimfos (IS)	**10.980**
Cocaine	13.356
Imipramine	13.536
Acetylsalicilic acid	ND
Bromazepam	ND
Caffeine	ND
Codeine	ND
Diazepam	ND
Diclofenac	ND
Fluoxetine	ND
Ibuprofen	ND
MDA	ND
MDMA	ND
Nortriptyline	ND
Paracetamol	ND

The bold values represent the retention times of the analytes; ND: not detected.

Optimization of HFμE

Desorption step

Ethyl acetate, acetone and hexane were evaluated as desorption solvents. Duplicate analysis with each solvent was performed following extraction of blank whole blood samples added with the analytes and internal standard. The medium peak area obtained for each analyte showed that most of them showed better response when desorption was performed with ethyl acetate.

Besides the desorption solvent, factors that influence the desorption efficiency include agitation mode and desorption time. These factors were also evaluated using a 2^2 factorial design. In this study, time had more impact on response when ultrasound was applied. Overall, 1 minute vortex desorption was more efficient than 5 minutes ultrasound desorption for all analytes, except for disulfoton. So, desorption was set to be carried out in ethyl acetate, through 1 minute vortex agitation, in order to improve analytical frequency.

Multivariate optimization – pH, agitation speed and salt addition

Optimization of the factors pH, agitation speed and salt addition were performed by multivariate mode. Multivariate optimization allows to visualize interaction effects between the factors, as opposed to univariate optimization.[41] For this purpose, a Box-Behnken design was performed (Table II). Box-Behnken designs are based in 3 levels fractional factorial designs. The study was performed with quintuplicate of central point, resulting in 17 experiments. The data obtained for all analytes were analyzed using the desirability function. The use of desirability allows the evaluation of all the analytes together. The response obtained for each analyte in each assay was converted to a desirability value between 0 and 1, as 0 was defined as 50% of the lowest area obtained for each analyte, and 1 as the highest area plus 50%. The calculated desirability values were submitted to a multiple linear regression and the pareto graph of the effects can be seen at Figure 2.

According to the graph, the only parameter with significant effect, by itself, is pH. As previously mentioned, pH must be adjusted to a value where analytes remain in non-ionized form, according to pKa.[35,37,38] As this study included both acidic and basic compounds, as well as non-ionic, with different values of pKa (Table III), an optimum pH value for all analytes would be impossible. Thus, a compromise condition should be found. The pH effect was positive, that is, higher the pH, higher the extraction efficiency. So, pH 6 was selected as the best pH to perform the extraction.

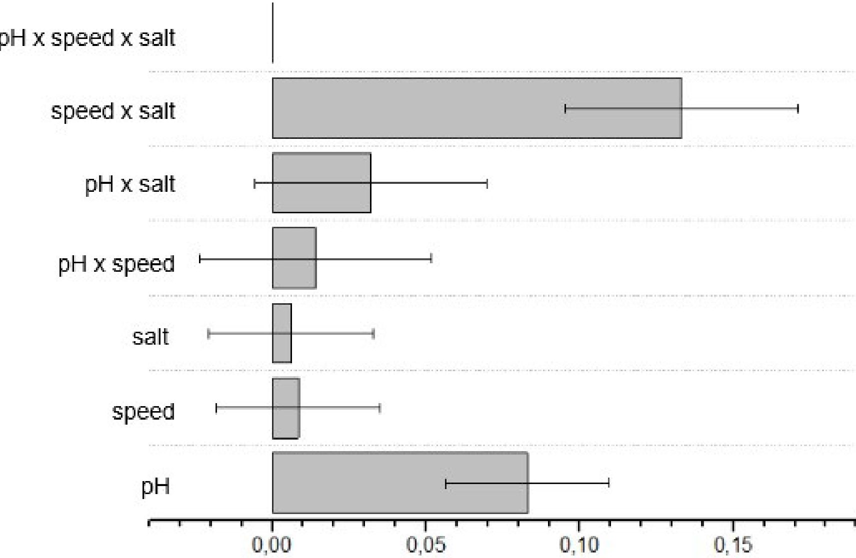


Figure 2. Pareto graph of the extraction effects – pH, agitation speed and salt addition.

Agitation speed was expected to show a positive effect since it improves analytes diffusion. However, no effect was observed, indicating that the lowest speed used was already high enough for this parameter. Salt addition can both increase or decrease extraction efficiency. In this case, no effect was observed for this parameter either.[30,35-37]

Although agitation speed and salt addition, by themselves, showed no effect in extraction efficiency, there's an interaction effect between both, which is also demonstrated by the pareto graph (Figure 2). To evaluate the best condition of these two factors, the response surface was plotted (Figure 3). The response surface shows that higher extraction efficiency is obtained at lower agitation (600 rpm) with no salt addition, or at higher agitation speed (1.000 rpm) and salt addition at 5% m/v concentration. This behavior occurs probably because the salt addition increases the viscosity of the donor phase, which difficult the analytes diffusion. Thus, higher agitation speed is needed to compensate for this negative impact. Between these two conditions of maximum response, the condition with agitation at 600 rpm and no salt addition was selected to minimize reagent consumption.

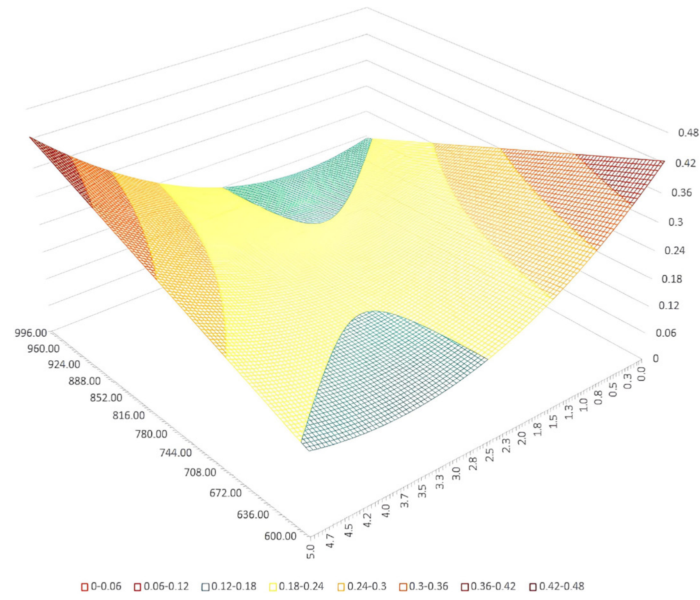


Figure 3. Response surface of the factors stirring speed and salt addition in the extraction.

Extraction time

After multivariate optimization, time univariate optimization was performed. Triplicate assays were performed at 10, 20, 30, 40, 50 and 60 minutes. The response of all analytes increased until 40 minutes of extraction. After this time, most of the compounds reach a plateau, indicating that equilibrium was achieved. Variation in analytes response that occurred after this time were all within the standard deviation. Therefore, 40 min was seen as the optimum time to perform analytes extraction.

CONCLUSION

Hollow fiber microextraction (HFμE) is a simple extraction technique based on other membrane-based extractions, like HF-LPME, but making it easier by eliminating the use of microsyringes and reducing manual steps that requires operator skill. It uses only few microliters of extraction solvent, in alignment with green chemistry techniques. In this work, the potential use of this technique in postmortem forensic toxicology was demonstrated by the optimization of an extraction procedure for the detection of selected pesticides in whole blood samples. Further work includes the validation of the developed method before application in real samples.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" [CNPq-Brazil, grant number 407875/2018-2].

REFERENCES

(1) Wyman, J. F. Principles and Procedures in Forensic Toxicology. *Clin. Lab. Med.* **2012**, *32* (3), 493–507. https://doi.org/10.1016/j.cll.2012.06.005

(2) Skopp, G. Postmortem Toxicology. *Forensic Sci. Med. Pathol.* **2010**, *6* (4), 314–325. https://doi.org/10.1007/s12024-010-9150-4

(3) Smith, M. P.; Bluth, M. H. Forensic Toxicology: An Introduction. *Clin. Lab. Med.* **2016**, *36* (4), 753–759. https://doi.org/10.1016/j.cll.2016.07.002

(4) Ramos, J. S. A.; Pedroso, T. M. A.; Godoy, F. R.; Batista, R. E.; de Almeida, F. B.; Francelin, C.; Ribeiro, F. L.; Parise, M. R.; de Melo e Silva, D. Multi-Biomarker Responses to Pesticides in an Agricultural Population from Central Brazil. *Sci. Total Environ.* **2021**, *754*. https://doi.org/10.1016/j.scitotenv.2020.141893

(5) de Araújo Nascimento, F.; Alves, A. A.; Nunes, H. F.; Miziara, F.; Parise, M. R.; de Melo e Silva, D. Cultivated Areas and Rural Workers' Behavior Are Responsible for the Increase in Agricultural Intoxications in Brazil? Are These Factors Associated? *Environ. Sci. Pollut. Res.* **2020**, *27* (30), 38064–38071. https://doi.org/10.1007/s11356-020-09988-3

(6) Bochner, R.; Freire, M. M. Analysis of deaths by intoxication that occurred in Brazil from 2010 to 2015 based on the Mortality Information System (SIM). *Ciência & Saúde Coletiva* **2020**, *25* (2), 761-772. https://doi.org/10.1590/1413-81232020252.15452018

(7) Peters, F. T.; Wissenbach, D. K.; Busardo, F. P.; Marchei, E.; Pichini, S. Method Development in Forensic Toxicology. *Curr. Pharm. Des.* **2018**, *23* (36), 5455-5467. https://doi.org/10.2174/1381612823666170622113331

(8) Vincenti, M.; Cavanna, D.; Gerace, E.; Pirro, V.; Petrarulo, M.; Di Corcia, D.; Salomone, A. Fast Screening of 88 Pharmaceutical Drugs and Metabolites in Whole Blood by Ultrahigh-Performance Liquid Chromatography-Tandem Mass Spectrometry. *Anal. Bioanal. Chem.* **2013**, *405* (2–3), 863–879. https://doi.org/10.1007/s00216-012-6403-y

(9) Jones, G. R. Postmortem Toxicology. In: Jickells, S.; Negrusz, A. (Eds). *Clarke's Analytical Forensic Toxicology*. Pharmaceutical Press, London, 2008, pp 191–217.

(10) Skopp, G. Preanalytic Aspects in Postmortem Toxicology. *Forensic Sci. Int.* **2004**, *142* (2–3), 75–100. https://doi.org/10.1016/j.forsciint.2004.02.012

(11) Manousi, N.; Samanidou, V. Green Sample Preparation of Alternative Biosamples in Forensic Toxicology. *Sustain Chem. Pharm.* **2021**, *20*, 100388. https://doi.org/10.1016/j.scp.2021.100388

(12) Campillo, N.; Gavazov, K.; Viñas, P.; Hagarova, I.; Andruch, V. Liquid-Phase Microextraction: Update May 2016 to December 2018. *Appl. Spectrosc. Rev.* **2019**, *55* (4), 307–326. https://doi.org/10.1080/05704928.2019.1604537

(13) Wille, S. M. R.; Lambert, W. E. E. Recent Developments in Extraction Procedures Relevant to Analytical Toxicology. *Anal. Bioanal. Chem.* **2007**, *388* (7), 1381–1391. https://doi.org/10.1007/s00216-007-1294-z

(14) He, Y.; Concheiro-Guisan, M. Microextraction Sample Preparation Techniques in Forensic Analytical Toxicology. *Biomed. Chromatogr.* **2019**, *33* (1), 1–12. https://doi.org/10.1002/bmc.4444

(15) Burato, J. S. S.; Medina, D. A. V.; de Toffoli, A. L.; Maciel, E. V. S.; Lanças, F. M. Recent Advances and Trends in Miniaturized Sample Preparation Techniques. *J. Sep. Sci.* **2020**, *43* (1), 202–225. https://doi.org/10.1002/jssc.201900776

(16) Jain, R.; Jain, B.; Chauhan, V.; Deswal, B.; Kaur, S.; Sharma, S.; A. S. Abourehab, M. Simple Determination of Dichlorvos in Cases of Fatal Intoxication by Gas Chromatography-Mass Spectrometry. *J. Chromatogr. B* **2023**, *1215*. https://doi.org/10.1016/j.jchromb.2022.123582

(17) Machado, S. C.; Souza, B. M.; Marciano, L. P. A.; Pereira, A. F. S.; de Carvalho, D. T.; Martins, I. A Sensitive and Accurate Vortex-Assisted Liquid-Liquid Microextraction-Gas Chromatography-Mass Spectrometry Method for Urinary Triazoles. *J. Chromatogr. A* **2019**, *1586*, 9–17. https://doi.org/10.1016/j.chroma.2018.11.082

(18) Shin, H. Trace-level Analysis of Polychlorinated Biphenyls, Organochlorine Pesticides and Polycyclic Aromatic Hydrocarbons in Human Plasma or Serum by Dispersive Liquid–Liquid Microextraction and Gas Chromatography–Tandem Mass Spectrometry. *Biomed. Chromatogr.* **2022**, *36* (6). https://doi.org/10.1002/bmc.5360

(19) Akramipour, R.; Golpayegani, M. R.; Sedighi, M.; Fattahi, F.; Fattahi, N. Determination of Common Organophosphorus Pesticides in the Blood of Children with Acute Leukaemia Using a Double-Solvent System as a Novel Extractant for Dispersive Liquid-Liquid Microextraction. *RSC Adv.* **2020**, *10* (73), 44736–44742. https://doi.org/10.1039/d0ra09303c

(20) Gallo, V.; Tomai, P.; Gherardi, M.; Fanali, C.; De Gara, L.; D'Orazio, G.; Gentili, A. Dispersive Liquid-Liquid Microextraction Using a Low Transition Temperature Mixture and Liquid Chromatography-Mass Spectrometry Analysis of Pesticides in Urine Samples. *J. Chromatogr. A* **2021**, *1642*. https://doi.org/10.1016/j.chroma.2021.462036

(21) Ramin, M.; Khadem, M.; Omidi, F.; Pourhosein, M.; Golbabaei, F.; Shahtaheri, S. J. *Development of Dispersive Liquid-Liquid Microextraction Procedure for Trace Determination of Malathion Pesticide in Urine Samples*. *Iran. J. Public Health* **2019**, *48* (10), 1893-1902. https://doi.org/10.18502/ijph.v48i10.3498

(22) Zhu, M.; Niu, Z.; Zhang, W.; Zhang, J.; Wen, Y. The Combination of Two Microextraction Methods Coupled with Gas Chromatography for the Determination of Pyrethroid Insecticides in Multimedia Environmental Samples: Air, Water, Soil, Urine and Blood. *Water and Environ. J.* **2020**, *34* (S1), 503–515. https://doi.org/10.1111/wej.12553

(23) Jouyban, A.; Farajzadeh, M. A.; Mogaddam, M. R. A. Dispersive Liquid–Liquid Microextraction Based on Solidification of Deep Eutectic Solvent Droplets for Analysis of Pesticides in Farmer Urine and Plasma by Gas Chromatography–Mass Spectrometry. *J. Chromatogr. B* **2019**, *1124*, 114–121. https://doi.org/10.1016/j.jchromb.2019.06.004

(24) Su, H.; Lin, H. H.; Su, L. J.; Lin, C. C.; Jiang, Z. H.; Chen, S. J.; Shiea, J.; Lee, C. W. Direct Immersion Solid-Phase Microextraction Combined with Ambient Ionization Tandem Mass Spectrometry to Rapidly Distinguish Pesticides in Serum for Emergency Diagnostics. *J. Food Drug Anal.* **2022**, *30* (1), 26–37. https://doi.org/10.38212/2224-6614.3399

(25) Zuluaga, M.; Yathe-G, L.; Rosero-Moreano, M.; Taborda-Ocampo, G. Multi-Residue Analysis of Pesticides in Blood Plasma Using Hollow Fiber Solvent Bar Microextraction and Gas Chromatography with a Flame Ionization Detector. *Environ. Toxicol. Pharmacol.* **2021**, *82*. https://doi.org/10.1016/j.etap.2020.103556

(26) Mafra, G.; Will, C.; Huelsmann, R.; Merib, J.; Carasek, E. A Proof-of-Concept of Parallel Single-Drop Microextraction for the Rapid and Sensitive Biomonitoring of Pesticides in Urine. *J. Sep. Sci.* **2021**, *44* (9), 1961–1968. https://doi.org/10.1002/jssc.202001157

(27) Ramin, M.; Omidi, F.; Khadem, M.; Shahtaheri, S. J. Combination of Dispersive Solid-Phase Extraction with Dispersive Liquid-Liquid Microextraction Followed by High-Performance Liquid Chromatography for Trace Determination of Chlorpyrifos in Urine Samples. *Int. J. Environ. Anal. Chem.* **2021**, *101* (6), 810–820. https://doi.org/10.1080/03067319.2019.1672670

(28) Pedersen-Bjergaard S.; Rasmussen, K. E. Liquid-Liquid-Liquid Microextraction for Sample Preparation of Biological Fluids Prior to Capillary Electrophoresis. *Anal. Chem.* **1997**, *71* (14), 2650–2656. https://doi.org/10.1021/ac990055n

(29) Esrafili, A.; Baharfar, M.; Tajik, M.; Yamini, Y.; Ghambarian, M. Two-Phase Hollow Fiber Liquid-Phase Microextraction. *TrAC, Trends Anal. Chem.* **2018**, *108*, 314–322. https://doi.org/10.1016/j.trac.2018.09.015

(30) Sharifi, V.; Abbasi, A.; Nosrati, A. Application of Hollow Fiber Liquid Phase Microextraction and Dispersive Liquid-Liquid Microextraction Techniques in Analytical Toxicology. *J. Food Drug. Anal.* **2016**, *24* (2), 264–276. https://doi.org/10.1016/j.jfda.2015.10.004

(31) López-López, J. A.; Mendiguchía, C.; Pinto, J. J.; Moreno, C. Application of Solvent-Bar Micro-Extraction for the Determination of Organic and Inorganic Compounds. *TrAC, Trends Anal. Chem.* **2019**, *110*, 57–65. https://doi.org/10.1016/j.trac.2018.10.034.

(32) Yamini, Y.; Rezazadeh, M.; Seidi, S. Liquid-Phase Microextraction – The Different Principles and Configurations. *TrAC, Trends Anal. Chem.* **2019**, *112*, 264–272. https://doi.org/10.1016/j.trac.2018.06.010

(33) Ide, A. H.; Nogueira, J. M. F. Hollow Fiber Microextraction: A New Hybrid Microextraction Technique for Trace Analysis. *Anal. Bioanal. Chem*. **2018,** *410*, 2911–2920. https://doi.org/10.1007/s00216-018-0971-4

(34) Ide, A. H.; Nogueira, J. M. F. Dual-Hollow Fiber Microextraction (Dual-HFμE) - Application for Monitoring Trace Levels of Organochlorine Pesticides in Real Matrices. *Int. J. Environ. Anal. Chem.* **2019**, *100* (12), 1402–1414. https://doi.org/10.1080/03067319.2019.1655006

(35) Alsharif, A. M. A.; Tan, G. H.; Choo, Y. M.; Lawal, A. Efficiency of Hollow Fiber Liquid-Phase Microextraction Chromatography Methods in the Separation of Organic Compounds: A Review. *J. Chromatogr. Sci.* **2017**, *55* (3), 378–391. https://doi.org/10.1093/chromsci/bmw188

(36) Dmitrienko, S. G.; Apyari, V. V.; Tolmacheva, V. V.; Gorbunova, M. V. Liquid–Liquid Extraction of Organic Compounds into a Single Drop of the Extractant: Overview of Reviews. *J. Anal. Chem.* **2021**, *76* (8), 907–919. https://doi.org/10.1134/S1061934821080049

(37) Pedersen-Bjergaard, S. Microextraction with Supported Liquid Membranes. In: Poole, C. F. (Ed). *Liquid-Phase Extraction, Handbooks in Separation Science*. Elsevier, 2019. Chapter 8, pp 241-263. https://doi.org/10.1016/B978-0-12-816911-7.00008-6

(38) He, Y. Recent Advances in Application of Liquid-Based Micro-Extraction: A Review. *Chem. Pap.* **2014**, *68* (8), 995–1007. https://doi.org/10.2478/s11696-014-0562-6

(39) PPDB: Pesticide Properties Database. https://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm#T

(40) PubChem Database. https://pubchem.ncbi.nlm.nih.gov

(41) Ferreira, S. L. C.; Bruns, R. E.; Ferreira, H. S.; Matos, G. D.; David, J. M.; Brandão, G. C.; da Silva, E. G. P.; Portugal, L. A.; dos Reis, P. S.; Souza, A. S.; dos Santos, W. N. L. Box-Behnken Design: An Alternative for the Optimization of Analytical Methods. *Anal. Chim. Acta* **2007**, *597* (2), 179–186. https://doi.org/10.1016/j.aca.2007.07.011