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A Highly Efficient Preconcentration/Clean-Up Method for Insecticides, Fungicides, and Herbicides Determination in Water Samples and Flour Samples by HPLC-DAD Using Water-Induced and Restricted-Access Supramolecular Microextraction

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A green supramolecular vortex-assisted microextraction employing a mixture of 1-decanol and tetrahydrofuran (THF) was developed for the simultaneous extraction of pesticides from different classes (insecticides, fungicides, and herbicides) from water and flour samples, followed by their determination in high-performance liquid chromatography with diode array detector (HPLC-DAD). The most favorable results were achieved at pH 6.0 in the presence of 4% (m/v) NaCl, utilizing a low volume of 1-decanol and THF (75 and 400 µL, respectively), a short vortex-assisted extraction time (120 s), and centrifugation time (120 s). These conditions yielded high preconcentration factors ranging from 50.1 to 90.3 and low limits of quantification (LOQs) ranging from 1.3 to 27.3 µg L^{-1}. The intraday (n = 10) and interday (n =1 0) precision, assessed as the percentage of relative standard deviation (RSD), varied from 4.4 to 12.4%. The optimized method was successfully applied to determine the pesticides in water and flour samples, with recoveries ranging from 81 to 111%.

Keywords: supramolecular solvent microextraction, pesticide compounds, water samples, flour samples

Introduction

Brazil has a productive area of more than 72 million hectares and a total volume of 312.5 million tons of grains estimated for the 2022/2023 harvest, 15% higher than in the past harvest.[1] For this motive, pesticide consumption in the country is notably high, and the approval status of pesticide-active ingredients differs significantly when compared to other agricultural nations.[2] In Brazil, herbicides represent the most used class, followed by insecticides and fungicides.[3]

The World Health Organization (WHO) and each country establish recommendations and guidelines for minimum water quality parameters, including maximum allowable pesticide levels, for consumption by their respective populations. These standards are formulated based on advancements in technical-scientific knowledge and draw from international experiences.[4] In Brazil, Agência Nacional de Vigilância Sanitária (Anvisa),[5] and the Ministry of Health[6] are responsible, respectively, for establishing the maximum residue limit (MRL) of pesticides in food and potable water. Conselho Nacional do Meio Ambiente (CONAMA) establishes the maximum admissible levels of chemical parameters in water bodies and the discharge of effluents.[7]

The legislation regarding the use of pesticides is different when comparing Brazil and other countries. For instance, prochloraz has its use banned in Brazil and the European Union but it is allowed in the United Kingdom. According to ANVISA's decision, the toxicological reassessment of the product did not meet the guidelines and safety requirements adopted by the agency and its monograph was in force until December 31, 2017.[5] Propanil and trifluralin, permitted in Brazil and Australia, are not permitted in the European Union,[8] and United States.[9] Among these pesticides, only trifluralin has a maximum limit allowed in potable water in Brazil (20 µg L^{-1}), while in the European Union, the individual value is set as 0.1 µg L^{-1} and the total is 0.5 µg L^{-1}. For foods, there is a discrepancy between the allowed values, as is noticed in the case of chlorantraniliprole (see MRLs in Supplementary Information (SI) section, Table S1).[5,8,9]

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Multiresidue methods have been generally used for determining several pesticides in different matrices,[10] and QuEChERS (quick, easy, cheap, effective, rugged and safe) method which uses acetonitrile or ethyl acetate as extraction solvent is the most common.[11-13]

Methods based on low consumption of extractor solvent derived from liquid-liquid microextraction methods, such as DLLME (dispersive liquid-liquid microextraction), DES (deep eutectic solvent), and supramolecular solvent (SUPRAs) have been increasingly adopted due to high preconcentration factors and because agree with principles of green analytical chemistry.[14]

SUPRAs are green water-immiscible solvents composed of amphiphile aggregates making them excellent candidates for the replacement of traditional toxic organic solvents, hexane, and chloroform in analytical extraction procedures from the aqueous medium. They are generated through sequential self-assembly of amphiphilic molecules, induced by changes in external stimuli, occurring on the molecular and nanoscales.[15]

Supramolecular solvents are excellent for the extraction of a wide range of organic pollutants, such as pesticides. These solvents establish different interactions with pesticides through ionic bonding, hydrogen bonding, and hydrophobic interaction, which explain the great performance in extraction procedures.[15,16] The high content of amphiphilic molecules self-assembly in the format of reversed micelles contains a high number of available binding sites for the extraction of pesticide residues using low volumes of the SUPRASs.[14,17,18] Moreover, SUPRAs present restricted access properties toward macromolecules, such as protein and carbohydrates, thereby their application in the analysis of food solid samples has also been successfully reported in the literature.[19]

Building upon the aforementioned considerations, this study focuses on the development of a novel, easy, highly improved, and environmentally sustainable supramolecular solvent-based microextraction method for different chemical classes of pesticides. Chlorantraniliprole (CAP, insecticide), kresoxim-methyl (KRE, fungicide), prochloraz (PRC, fungicide), propanil (PRP, herbicide) and trifluralin (TRI, herbicide) were chosen because they present different status of approval or are banned in Brazil and around of world legislations.

Experimental

Reagents and solutions

All reagents were analytical or high-performance liquid chromatography (HPLC) grade. Methanol (99.9%), tetrahydrofuran (THF, ≥ 99.9%), 1-decanol (≥ 98.0%), sodium chloride (NaCl, ≥ 99.5%), sodium nitrate ($NaNO_3$, ≥ 99.9%) and sodium sulfate (Na_2SO_4, ≥ 99.9%), acetic (CH_3COOH, ≥ 99.5%), phosphoric (H_3PO_4, ≥ 85%) and boric (H_3BO_3, ≥ 99.5%) acid were purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). The pesticide standards were all acquired from Sigma-Aldrich (Saint Louis, Missouri, USA).

Stock solutions of pesticides (100 mg L^{-1}) were prepared in methanol, and stored in amber flasks in a freezer (–20 °C). Working solutions were prepared freshly and diluted with ultrapure water (18.2 MΩ cm) collected from a purification system ELGA PURELAB (Woodridge, Illinois, USA).

Instruments

Chromatographic analyses were performed on an HPLC system (Shimadzu Prominence, Tokyo, Japan) composed of an LC-20AT pump, CTO-20A column oven, a degasser system DGU20A, and a 7725i manual injector with a 20 µL loop, (Rheodyne, Rohnert Park, California, USA). Graphical representation and statistical analysis were made by the software Origin Pro 8 SR0 v8.0724(B724)[20] and Statsoft Statistica 7.0 software,[21] respectively. Solutions and pH samples were measured with a Metrohm 827 pH mobile digital pH meter (Ionenstrasse, Herisau, Switzerland). Vortex agitator SCILOGEX MX-S (Rocky Hill, Connecticut, USA) and a centrifuge QUIMIS®0222T2 (Diadema, São Paulo, Brazil) were used to blend fluids quickly to assist the supramolecular solvent-based microextraction and phase separation process, respectively. The supramolecular rich phase formed was removed using an HPLC syringe Hamilton 50.0 µL model 1705 N (Reno, Nevada, USA).

HPLC analysis

Chromatographic separation was carried out using a C18 column (Phenomenex, 250 mm × 4.5 mm, particle size 5 µm) and a guard column (Phenomenex, 4.0 mm × 30 mm internal diameter, 5 µm in particle size) (Torrance, California, USA), oven temperature of 30 °C with a mobile phase consisting of a binary gradient of methanol and water (MeOH: H_2O, v/v) at a flow rate of 1.0 mL min^{-1}. The gradient was carried out as MeOH:H_2O (70:30, v/v), 0.00 min; MeOH: H_2O (from 70:30 to 80:20, v/v), 0.01-9.00 min; MeOH: H_2O (80:20, v/v), 9.00-12.00 min; MeOH: H_2O (from 80:20 to 90:10, v/v), 12.00-15.00 min; MeOH: H_2O (90:10, v/v), 15.00-21.00 min.

The pesticide retention times were 5.8 min for chlorantraniliprole, 7.5 min for propanil, 10.2 min for

kresoxim-methyl, 11.6 min for prochloraz, and 19.1 min for trifluralin. The wavelengths were set as 200 nm for kresoxim-methyl, prochloraz, and trifluralin, 206 nm for chlorantraniliprole, and 210 nm for propanil, using a diode array detector (DAD). All chromatographic area values were processed utilizing the software LabSolutions® LC solution version 1.25 (Shimadzu, Tokyo, Japan).

Supramolecular solvent-based microextraction procedure

Ten milliliters of the standard solution of five pesticides at pH 6.0 buffered with 0.01 mol L^{-1} Britton-Robinson (BR) containing 4% (m/v) NaCl were placed in a 20.0 mL screw-capped glass tube. Then, 75 µL of 1-decanol and 400 µL of THF were added into the tube. Afterward, the mixture was vortex-assisted stirred for 120 s. The phase separation of the supramolecular solvent was accelerated by centrifugation at 2000 rpm for 120 s. Finally, the volume of the enrichment phase, at the top of the tube, was removed using an HPLC syringe and injected into the chromatographic system. The chemical structure of pesticides and supramolecular solvent-based microextraction procedure is illustrated in Figure 1.

The effect of experimental variables on the extraction efficiency of pesticides was investigated by univariate method. The influence of pH range (4.0-10.0), the volume of 1-decanol (50-150 µL), the volume of THF (100-2000 µL), salting out effect (0.0-5.0% NaCl, m/v), salt type (NaCl, $NaNO_3$, and Na_2SO_4), concentration buffer (0.1-0.005 mol L^{-1}) buffer type (Britton-Robinson buffer and phosphate buffer), vortex stirring time (30-150 s) and centrifuge time (120-900 s) were investigated in the respective order (the detailed experimental variables are shown in Table S2, SI section).

The concentrations for chlorantraniliprole and propanil were set as 300 µg L^{-1}, kresoxim-methyl, and prochloraz were set as 100 µg L^{-1}, and trifluralin was set as 600 µg L^{-1}.

Analytical parameters procedure

In order to improve the detectability of the proposed method, a preconcentration volume of 10.0 mL, under optimized conditions, the analytical performance of the method was evaluated by linear regressions, preconcentration factor (PF), limits of quantification (LOQ) and detection (LOD), inter-intraday precision. The linear regressions were evaluated by analysis of variance (ANOVA) including the LOQ as the first concentration in the analytical curve (in triplicate), after subjecting the standard solutions of pesticides to the microextraction procedure. The LOD and LOQ were determined as 3SD/m and 10SD/m, respectively, where SD is the standard deviation of ten blank measurements and m is the slope of the analytical curve with supramolecular microextraction preconcentration.[22] The PF was calculated by the ratio of slopes of the linear regressions with and without the preconcentration step. Interday (n = 10) and intraday (n = 10) precision were calculated with two standard solutions containing chlorantraniliprole and propanil: 75 and 225 µg L^{-1}; kresoxim-methyl and prochloraz: 25 and 75 µg L^{-1}; trifluralin: 150 and 450 µg L^{-1} and the relative standard deviations (RSD) were determined.

Sample collection, preparation and preservation

Water samples from lake and river were collected from three different streams located in the cities of Londrina

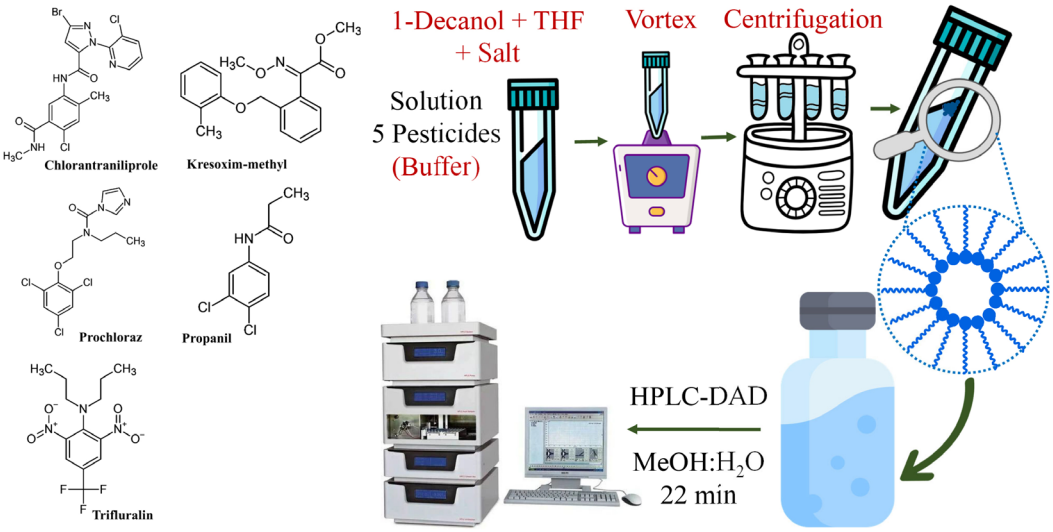

Figure 1. Chemical structures of chlorantraniliprole, kresoxim-methyl, prochloraz, propanil and trifluralin and schematic process of self-assembly and coacervation in the supramolecular microextraction of pesticides.

(Igapó Lake, coordinates: 23°19'15.2"S 51°10'54.9"W), Apucarana (Schmidt Lake, coordinates: 23°32'12.2"S 51°25'39.9"W) and Medianeira (Sol e Ouro River, coordinates: 25°20'37.7"S 54°05'47.1"W) in Paraná State, Brazil. These cities are located in regions known for their agricultural production. All samples were collected in dark glass containers and adjusted to pH 2.00 by adding concentrated sulfuric acid. Then, they were filtered through 0.45 µm Nylon® filters (GVS Filter Technology, Morecambe, United Kingdom) to remove suspended particles and stored in the refrigerator under light protection until analysis. Before supramolecular microextraction, using a preconcentration volume of 10.0 mL (n = 3), the pH was adjusted to 6.0 with 0.01 mol L^{-1} Britton-Robinson (BR) buffer containing 4% (m/v) NaCl and spiked with known amounts of pesticides.

The flour samples (oat, wheat, and rice) were purchased from local supermarkets in Londrina (Brazil) stored in their original containers, and kept according to the packaging recommendations. 6 g of the flour samples were mixed with 300.0 mL of water-buffered at pH 6.0 with Britton-Robinson containing NaCl in overnight. After the period, the samples were filtered, and the supernatant was slowly evaporated at 60 °C until a volume of 10.0 mL was obtained. Afterward, the supernatant was subjected to supramolecular microextraction under optimal conditions. The mixture was vortex shaken for 120 s and then centrifuged (2000 rpm) for 120 s to complete supramolecular solvent separation. The solvent was withdrawn transferred to microtubes and analyzed by HPLC-DAD. To assess the accuracy of the method, samples were spiked with a known amount of analytes and subjected to the proposed method.

Results and Discussion

Influence of pH on the microextraction

The pH value of the sample plays an important role in the coacervation and dispersion of supramolecular micelles in solution, as well as influences the ionization of pesticides. In liquid-liquid microextraction-based methods, molecules in neutral form result, in general, in higher extraction efficiency, as their solubility in water is decreased, with a consequent increase in the partitioning coefficient.[23] Thus, the extraction efficiency is explained bearing in mind the acid-base ionization properties of pesticides, solubility in water, and partitioning phenomena based on Log Kow, as depicted in Table 1. The pesticides in this study can be ionized according to their pK_a, except for trifluralin.[24,25]

The influence of pH on the extraction performance was investigated in the range of 4.0-10.0. According to Table 1, the pesticides are found in their neutral form, and for this reason, the analytical signals at the different pH ranges were practically unchanged (Figure 2). Since pH 6.0 is closer to the neutral pH of freshwater, this value was chosen for further experiments.

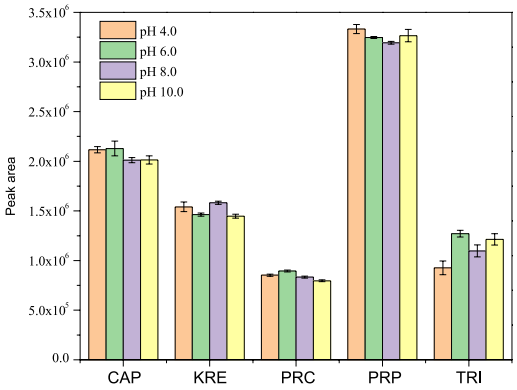

Figure 2. Effect of pH on the supramolecular microextraction of pesticides (n = 3). Experimental conditions: 10.0 mL of pesticide solution, BR buffer 0.01 mol L^{-1}, no salt, 100 µL of 1-decanol, 500 µL of THF, vortex for 120 s and 10 min of centrifugation. Concentration of pesticides: CAP and PRP 300 µg L^{-1}, KRE and PRC 100 µg L^{-1} and TRI 600 µg L^{-1}.

Volume of extractor 1-decanol and disperser THF

The chemical structure of the amphiphile used as an extractor has a strong influence on the microextraction

Table 1. Physical and chemical properties of pesticides

Pesticide[a]	Molecular formula[a]	Pesticide type	Chemical group	Molecular weight / (g mol^{-1})	Log K_{ow}[b]	Solubility in water[b] / (mg L^{-1}, 20 °C)	pK_a[b]	Retention time / min	Wavelength / nm
Chlorantraniliprole (CAP)	$C_{18}H_{14}BrCl_2N_5O_2$	insecticide	anthranilamide	483.1	2.86	0.88	1.58 and 13.79	5.8	206.0
Kresoxim methyl (KRE)	$C_{18}H_{19}NO_4$	fungicide	strobilurin	313.35	3.4	2.0	–0.96	10.2	200.0
Prochloraz (PRC)	$C_{15}H_{16}Cl_3N_3O_2$	fungicide	imidazole carboxamide	376.7	4.12	34.4	2.55	11.6	200.0
Propanil (PRP)	$C_9H_9Cl_2NO$	herbicide	anilide	218.08	3.3	130.0	13.90	7.5	210.0
Trifluralin (TRI)	$C_{13}H_{16}F_3N_3O_4$	herbicide	dinitroaniline	335.28	5.07	0.22	no dissociation	19.1	200.0

[a]Anvisa;[5] [b]data from Roberts and Hutson[24] and Roberts.[25]

process and, consequently, on preconcentration. As amphiphiles are capable of establishing different types of bonds with organic compounds. The extraction efficiency depends on the number of amphiphiles and binding energies (ionic > hydrogen bonding > dipole-dipole > dipole-induced dipole > dispersion) in the supramolecular solvent.[26]

The supramolecular solvent used was based on 1-decanol reverse micelles dispersed in a THF: water continuous phase. Therefore, the volume of 1-decanol was studied in the range of 50-150 µL (Figure 3). It is observed that the smallest volume evaluated (50 µL) presented, in general, the highest analytical signal. However, when dealing with small volumes (50 µL), the precision becomes more difficult to achieve. Thus, for further optimizations, 75 µL of 1-decanol volume was used as a compromise between analytical signal and precision.

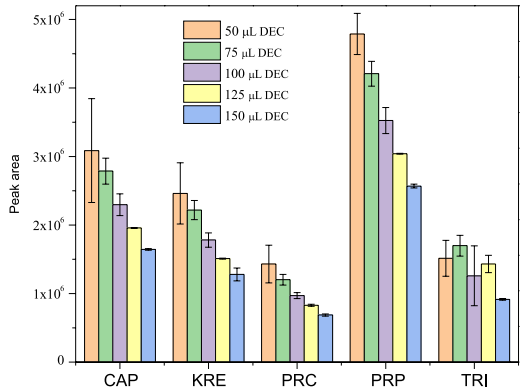

Figure 3. Effect of volume of 1-decanol on the supramolecular microextraction of pesticides (n = 3). Experimental conditions: 10.0 mL of pesticide solution, pH 6.0, BR buffer 0.01 mol L^{-1}, no salt, 500 µL of THF, vortex for 120 s and 10 min of centrifugation. Concentration of pesticides: CAP and PRP 300 µg L^{-1}, KRE and PRC 100 µg L^{-1} and TRI 600 µg L^{-1}

The influence of THF volume was in the range of 100-2000 µL. THF induces dispersion of the amphiphiles and makes it easier the self-assembly of micelles, thereby solvating the hydrocarbon chains.[27] Consequently, when the volume of THF is relatively small compared to the volume of 1-decanol, the performance of THF for dispersing the 1-decanol is drastically decreased, resulting in a reduced extraction of pesticides. On the other hand, an excessive THF volume can lead to a better interaction of pesticides with THF/water and low extraction efficiency by 1-decanol.[28] As one can see in Figure 4, slightly higher analytical signals for all pesticides were achieved using 400 µL of THF. Therefore, this volume was adopted for further experiments.

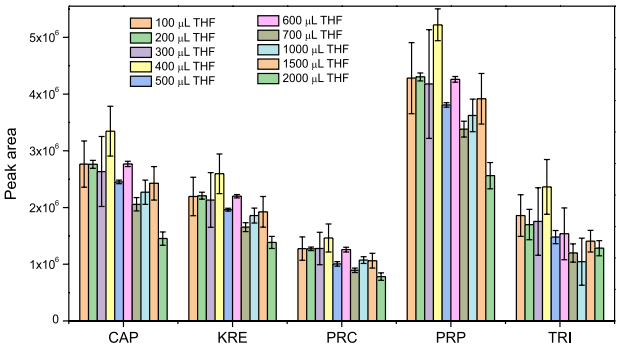

Figure 4. Effect of volume of THF on the supramolecular microextraction of pesticides (n = 3). Experimental conditions: 10.0 mL of pesticide solution, pH 6.0, BR buffer 0.01 mol L^{-1}, no salt, 75 µL of 1-decanol, vortex for 120 s and 10 min of centrifugation. Concentration of pesticides: CAP and PRP 300 µg L^{-1}, KRE and PRC 100 µg L^{-1} and TRI 600 µg L^{-1}.

Salting out effect

The presence of salt in the extracting medium is carried out to increase the recovery of the extraction by the salting-out effect. The presence of electrolyte salt in the aqueous sample increases its ionic strength, reducing the solubility of the analyte in the aqueous solution, and thus making the partitioning of target analytes easier.[29] Sodium chloride (NaCl), a readily accessible and commonly used substance in the laboratory, was selected as the salting-out agent. The investigation aimed to determine whether variations in the weight/volume ratio of NaCl result in improved response values.

Figure 5a illustrates an increase in response signal with an increase in the proportion of NaCl from 1 to 4%, followed by a decline at 5% (m/v) of NaCl. It is worth noting that, for the pesticides chlorantraniliprole, propanil, and trifluralin, the response values obtained without the presence of NaCl are greater than those achieved with the addition of 1% (m/v) NaCl. This suggests that the extraction method remains effective even without the salting-out effect. Nevertheless, it was noted that the absence of salt makes the phase separation process more difficult, resulting in low precision in the collection of the rich phase. Therefore, a 4% (m/v) NaCl solution was adopted as the optimum condition in the microextraction method.

The impact of the different salts on extraction efficiency was further examined by introducing sodium salts with varying anions, specifically NaCl, $NaNO_3$, and Na_2SO_4. These salts are all categorized as strong electrolytes, meaning they completely dissociate in aqueous solutions. It can be seen in Figure 5b that there is no difference between the results obtained for each pesticide with the different types of salts, despite the difference in charge between the monovalent anions Cl^- and NO_3^- and the divalent anion SO_4^{2-}. Although electrolytes containing divalent anions are more effective in promoting salting of the aqueous solution than those containing monovalent anions due to greater competition for water hydration,[30,31] which leads to

a decrease in the solubility of pesticides, this was not the result observed and, therefore, NaCl continued to be used in the sequence of experiments.

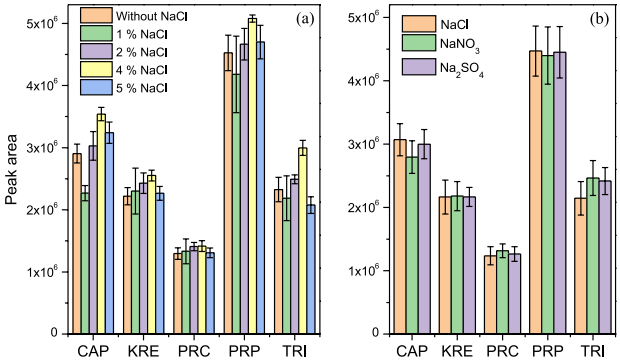

Figure 5. Effect of salting out on the supramolecular microextraction of pesticides (n = 3). Experimental conditions: 10.0 mL of pesticide solution, pH 6.0, BR buffer 0.01 mol L^{-1}, 75 µL of 1-decanol, 400 µL of THF, vortex for 120 s and 10 min of centrifugation. Concentration of pesticides: CAP and PRP 300 µg L^{-1}, KRE and PRC 100 µg L^{-1} and TRI 600 µg L^{-1}

Influence of buffer solution

The BR buffer concentration was evaluated from 0.005 to 0.1 mol L^{-1} and the results are shown in Figure S1 (SI section). The concentration of 0.01 mol L^{-1} presents the best response values, corroborating its use as a buffer concentration. The type of buffer used was also evaluated, considering the working pH of 6.0 and the concentration of 0.01 mol L^{-1}. For this, BR buffer and phosphate buffer were employed. Results are shown in Figure S2 (SI section). The results obtained were similar for all pesticides, thus 0.01 mol L^{-1} BR buffer was maintained as the best condition.

Influence of the vortex stirring

Vortex-assisted microextraction accelerates the spontaneous formation of the supramolecular solvent in the solution. The influence of time extraction was studied varying from 30 to 150 s. As evidenced in Figure S2, the results indicate that 120 s was required to reach extraction equilibrium. For the longest extraction time, 150 s, the peak areas decreased (except for prochloraz), due to a possible restriction of supramolecular phase formation. The increase in the vortex agitation time increases the contact time, which in turn accelerates the mass transfer of the target analytes to the extractor, however, after 120 s the extraction efficiency decreased, due to the back diffusion of the analytes in the sample solution.[29]

Influence of centrifugation time

This crucial step affects the quality and quantity of rich phase recovery and speeds up the isolation and collection process after extraction. The effects of centrifugation time were investigated at a centrifugation force of 2000 rpm. The centrifugation time varied in the range of 120 to 900 s (2 to 15 min), and it is observed in Figure S3 (SI section) that there is no increase in the chromatographic signal with the increase in the centrifugation time. In addition, for chlorantraniliprole and prochloraz, 120 s is the time in which the best chromatographic signal is obtained, thereby this centrifugation time was chosen as the best condition.

Figure 6 shows the chromatograms before and upon implementation of the preconcentration method under optimized conditions, which demonstrates the greatly improved signal intensity of chromatographic peaks.

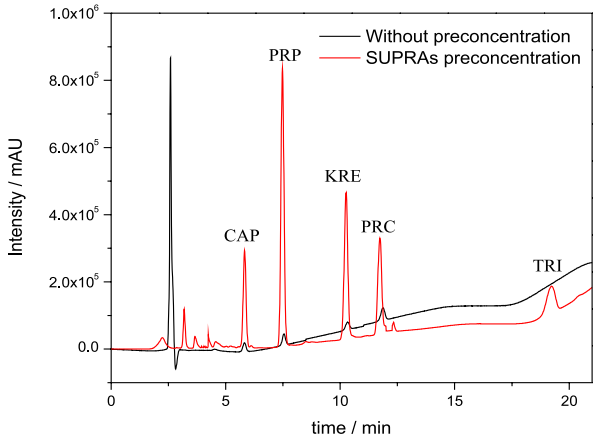

Figure 6. Chromatograms of 500 µg L^{-1} solution pesticides without preconcentration (black line) and submitted to supramolecular microextraction (red line) by preconcentrating 10.0 mL of solution. Conditions pH 6.0, BR buffer 0.01 mol L^{-1}, 75 µL of 1-decanol, 400 µL of THF, 4% (m/v) NaCl, vortex for 120 s and 120 s of centrifugation. Concentration of pesticides: CAP and PRP 300 µg L^{-1}, KRE and PRC 100 µg L^{-1} and TRI 600 µg L^{-1}

Analytical performance of the method

Table 2 shows the analytical parameters obtained by the proposed method for the five pesticides. The obtained linear regressions on the pesticides were statistically evaluated by one-way ANOVA with a confidence level of 95%. The MS_R/MS_{res} ratios defined by F_{cal} were much higher than F_{tab} (4.49), indicating that the linear models seem to be the most appropriate to describe the linear correlation between intensity and concentrations in the linear ranges. The $MS_{lackoffit}/MS_{pe}$ ratios (F-values) were lower than the F_{tab} value of 4.30, indicating the absence of lack-of-fit of the linear models.[32] It is worth emphasizing that analytical curves were previously constructed to obtain the theoretical LOD and LOQ. However, to check the practical feasibility of LOQ, i.e., if they are into the linear analytical curve without losses of linearity, standard solutions of pesticides

at LOQ concentration were prepared, subjected to the preconcentration step, and inserted as the first concentration of analytical curve, as shown in Figures S4a-S4e. Therefore, the data of linear regressions shown in Table 2 contain the LOQ as the first concentration of the analytical curve.

The obtained LOQ values were lower than the MRL for all pesticides established by ANVISA (Brazil), USEPA (United States), and European Union (Table S1). The PF values range from 50.1 to 90.3. The precision in terms of intra and interday ranged from 3.6 and 9.0%, which denotes the high precision of the proposed method.

The analytical performance of the method was compared to previously published methods for the determination of five pesticides in HPLC-DAD, as shown in Table 3. The supramolecular method stands out due to its low sample consumption, wide linear range, and satisfactory enrichment factor. The primary advantage of this method, in comparison to others, is its ability to achieve a limit of detection for pesticide determination that is close to the maximum values allowed by the Brazilian Ministry of Health, the European Union, and the USEPA (as detailed in Table S1) without the need for expensive or dangerous organic solvents, such as acetonitrile,[33-36] ethyl acetate,[37] dichloromethane[38] or mixtures of solvents such as ethyl acetate and hexane[39] and ethyl acetate and dichloromethane[36] used in other studies.

Most of the methods make use of QuEChERS,[33-35,37] but traditional methods also are employed such as matrix solid phase dispersion (MSPD) using alumina,[39,40] solid phase extraction (SPE),[36] liquid-liquid extraction (LLE),[41] DLLME,[42] or two associated treatment methods, such as solvent extraction followed by cleanup with silica gel[43] and LLE followed for SPE.[37] A unique study employs ultrasound-assisted DES dispersive liquid-phase microextraction.[44]

The supramolecular method has also proven to be both rapid and simple, utilizing only a vortex and a centrifuge in its process, thereby increasing analytical frequency and making it user-friendly. When compared to traditional methods, such as LLE and SPE, the obtained limits of detection and quantification are notably lower, underscoring how supramolecular microextraction enhances the sensitivity of classical analytical techniques.

The environmental friendliness of the proposed supramolecular microextraction method was evaluated using an innovative and recent tool named AGREE (Analytical GREEnness).[45] The status of each aspect is indicated by a color scheme ranging from green to yellow to red. In addition, an overall score between 0 and 1 is assigned. A score closer to 1 indicates greener, and the score decreases by assigning penalty points based on the 12 principles of green analytical chemistry. The output is a clock-like graph showing the total score and the color in the center (Figure S5, SI section).

The proposed method has a total score of 0.64 and a predominant color of green, which means that it has a low environmental impact and can be considered a green method. Multiresidues determined within 1 h are preferable to methods that use only one analyte at a time (Principle 8) and the sampling procedure requires only a few steps and no derivatization step (Principles 4 and 6). Additionally, the supramolecular microextraction method makes use of low sample volume and low solvent consumption, resulting in very low production of waste (Principles 2, 5, and 7) and low toxicity and risks to the user (Principles 11 and 12). However, the instrument used in the analysis of HPLC-DAD resulted in a yellow color (Principle 9). Yellow and red colors (Principles 1 and 3) were entered for the sampling procedure and positioning of the analytical instrument, as the sample was pretreated before injection for HPLC-DAD. Finally, the red color for the reagents (Principle 10) was based on the consideration that the solvent (THF) is not from bio-based sources, which offers better sustainability.

Analysis of environmental and food samples

To investigate the reliability of the proposed method for analysis of environmental and food samples, water

Table 2. Analytical parameters by supramolecular microextraction for the five pesticides

Pesticide	Linear range / (μg L^{-1})	Linear regression equation	R^2	*F*-value	LOD / (μg L^{-1})	LOQ / (μg L^{-1})	PF	Precision intraday[a] (RSD) / %		Precision interday[a] (RSD) / %	
CAP	1.9-300.0	$y = 1.0 \times 10^{10}x - 5.2 \times 10^{4}$	0.9907	1.68	0.6	1.9	87.5	4.7	4.5	4.4	3.6
KRE	4.3-175.0	$y = 2.1 \times 10^{10}x + 1.6 \times 10^{4}$	0.9964	0.91	1.3	4.3	84.9	8.7	8.0	6.3	8.3
PRC	5.7-175.0	$y = 1.7 \times 10^{10}x + 5.4 \times 10^{4}$	0.9944	0.20	1.7	5.7	74.9	5.6	9.0	7.6	9.0
PRP	1.3-525.0	$y = 1.1 \times 10^{10}x + 1.4 \times 10^{5}$	0.9922	2.74	0.4	1.3	90.3	7.6	8.4	6.0	6.9
TRI	27.3-1050.0	$y = 4.4 \times 10^{9}x - 1.1 \times 10^{5}$	0.9974	0.59	8.2	27.3	50.1	7.2	4.1	7.6	4.3

[a]Concentration analyzed in the precision studies: CAP: 75 and 225 μg L^{-1}; KRE: 25 and 75 μg L^{-1}; PRC: 25 and 75 μg L^{-1}; PRP: 75 and 225 μg L^{-1}; TRI: 150 and 450 μg L^{-1}. PF: preconcentration factor; LOD: limit of detection; LOQ: limit of quantification; RSD: relative standard deviation; CAP: chlorantraniliprole; KRE: kresoxim-methyl; PRC: prochloraz; PRP: propanil; TRI: trifluralin.

Table 3. Comparison of literature methods and the present method for chlorantraniliprole, kresoxim-methyl, prochloraz, propanil and trifluralin determination utilizing HPLC-DAD

Analyte	Preconcentration technique	Sample volume	time	Solvent volume	LOD / (μg L^{-1})	LOQ / (μg L^{-1})	PF	Sample application	Reference
CAP	QuEChERS		60 s	15.0 mL ethyl acetate	20.0	60.0		grape	37
CAP	ASE - cleanup with 25 g of silica gel			140.0 mL ethyl acetate: petroleum ether (1:25)	10.0	–		tobacco	43
CAP	SUPRAs	10.0 mL	120 s	75.0 μL of 1-decanol	0.6	1.9	87.5	water and cereal flours	this work
KRE	LLE	10.0 mL	120 s	30.0 mL of HCl 0.002 mol L^{-1} and 10.0 mL of ethyl ether	362.0	–		grape and wine	41
KRE	QuEChERS		180 s	10.0 mL acetonitrile with 1% acetate	5.0	10.0		orange (peel and pulp)	33
KRE	MSPD (alumina)			20.0 mL of cyclohexane/ ethyl acetate (1:1)	50.0	100.0		açai	39
KRE	QuEChERS		60 s	10.0 mL of acetonitrile with 1% acetic acid	10.0	50.0		apple	34
KRE	QuEChERS		60 s	10.0 mL of acetonitrile with 1% acetic acid	1.0	3.0		water and fish	35
KRE	SUPRAs	10.0 mL	120 s	75.0 μL of 1-decanol	1.3	4.3	84.9	water and cereal flours	this work
PRC	UA-SDES-DLPME	5.0 mL	9 min	67.0 μL of DES	8.40	25.0		juices and teas	44
PRC	SUPRAs	10.0 mL	120 s	75.0 μL of 1-decanol	1.7	5.7	74.9	water and cereal flours	this work
PRP	MSPD (alumina)			12.0 mL of ACN	5.0	16.0		rice	40
PRP	SPE	200.0 mL	40 min	2.0 mL ethyl acetate-dichlomethane mixture (9:1 v/v)	1.0	3.0		water	36
PRP	DLLME	5.0 mL		103.0 μL of carbon disulfide	0.03	–	91	water	42
PRP	SUPRAs	10.0 mL	120 s	75.0 μL of 1-decanol	0.4	1.3	90.3	water and cereal flours	this work
TRI	LLE and SPE	15.0 mL of MeOH		3.0 mL of CH_2Cl_2	1.0	–		juices	38
TRI	SUPRAs	10.0 mL	120 s	75.0 μL of 1-decanol	8.2	27.3	50.1	water and cereal flours	this work

CAP: chlorantraniliprole; KRE: kresoxim-methyl; PRC: prochloraz; PRP: propanil; TRI: trifluralin; QuEChERS: quick, easy, cheap, effective, rugged and safe; ASE: accelerated solvent extraction; LLE: liquid-liquid extraction; MSPD: matrix solid phase dispersion; UA-SDES-DLPME: ultrasound-assisted DESs:DLME by solidifying DESs-rich phase; DLLME: dispersive liquid-liquid microextraction; SUPRA: supramolecular solvent.

samples (lake and river) and wheat, oat, and rice flours were subjected to the preconcentration method (Table 4). The water samples were collected in Medianeira, Apucarana, and Londrina in the state of Paraná, Brazil. The flour samples were purchased from local markets in the city of Londrina, Brazil.

The recovery was used to prove the accuracy of the obtained data by the proposed sample preparation method. Three levels of a known amount of the standard were added to the water based on the limit of quantification obtained in the analytical performance of the method and recovery values were determined. For flour samples, one level of a known amount of the standard according to the MRL permitted by the European Union and recoveries values were determined. As observed, recoveries ranging from 82 to 111% were obtained, within the acceptable recovery range of 60 to 115%.[46,47] The results demonstrate the accuracy of the developed method for the extraction and determination of pesticides in water and flour samples. It must be pointed out that although pesticides were not detected in the samples, improvements in the sensibility of the method can be achieved by increasing the volume of the sample as demonstrated by our recent study.[18]

Figure S6 (SI section) shows the chromatogram of the two limits of quantification of the method for spiked river water samples. The proposed method application is

Table 4. Analysis of river and lake water samples and flours and recoveries values (n = 3)

Analyte	Concentration added / (μg L^{-1})			Concentration found / (μg L^{-1})			Recovery / %		
Sample location: Schmidt Lake (Apucarana-PR)									
CAP	4.0	6.0	8.0	3.7 ± 0.1	5.8 ± 0.1	8.1 ± 0.3	93	96	101
KRE	9.0	13.0	17.0	8.4 ± 0.2	11.7 ± 0.8	16.6 ± 1.2	93	90	98
PRC	11.0	17.0	23.0	11.9 ± 0.7	16.8 ± 0.6	24.3 ± 1.8	108	99	106
PRP	3.0	4.0	5.0	2.7 ± 0.4	3.9 ± 0.3	5.5 ± 0.6	89	98	109
TRI	55.0	82.0	109.0	55.7 ± 3.1	85.5 ± 3.0	113.1 ± 7.4	101	104	104
Sample location: Sol e Ouro River (Medianeira-PR)									
CAP	4.0	6.0	8.0	3.9 ± 0.3	5.4 ± 0.2	6.7 ± 0.4	98	90	84
KRE	9.0	13.0	17.0	9.3 ± 1.2	12.2 ± 0.7	14.9 ± 0.6	103	94	88
PRC	11.0	17.0	23.0	10.4 ± 1.8	17.1 ± 0.7	23.5 ± 0.9	94	101	102
PRP	3.0	4.0	5.0	2.4 ± 0.1	3.3 ± 0.6	5.2 ± 0.5	82	82	104
TRI	55.0	82.0	109.0	52.8 ± 4.3	83.5 ± 2.7	93.7 ± 2.3	96	102	86
Sample location: Igapó lake (Londrina-PR)									
CAP	4.0	6.0	8.0	4.1 ± 0.3	5.8 ± 0.1	7.3 ± 0.3	103	97	91
KRE	9.0	13.0	17.0	8.3 ± 1.0	10.6 ± 0.9	15.2 ± 0.3	92	81	90
PRC	11.0	17.0	23.0	9.7 ± 0.9	17.5 ± 0.7	22.1 ± 0.6	88	103	96
PRP	3.0	4.0	5.0	2.5 ± 0.6	3.5 ± 0.3	5.1 ± 0.5	82	87	102
TRI	55.0	82.0	109.0	60.9 ± 0.3	83.4 ± 3.9	108.8 ± 4.9	111	102	100
Sample: oat flour									
CAP		12.0			10.7 ± 0.3			90	
KRE		48.0			41.2 ± 1.3			86	
PRC		120.0			97.7 ± 4.6			81	
PRP		6.0			5.1 ± 0.5			85	
TRI		30.0			28.4 ± 0.7			95	
Sample: wheat flour									
CAP		12.0			10.2 ± 0.5			85	
KRE		90.0			85.1 ± 2.0			95	
PRC		18.0			15.9 ± 0.7			89	
PRP		6.0			5.4 ± 0.3			90	
TRI		30.0			28.5 ± 0.3			95	
Sample: rice flour									
CAP		240.0			214.0 ± 4.6			89	
KRE		6.0			5.3 ± 0.1			89	
PRC		18.0			15.0 ± 0.5			83	
PRP		6.0			5.1 ± 0.2			87	
TRI		30.0			27.9 ± 0.2			93	

CAP: chlorantraniliprole; KRE: kresoxim-methyl; PRC: prochloraz; PRP: propanil; TRI: trifluralin.

quite simple, rapid, and environmentally friendly by not requiring high amounts of organic solvent. It also enables direct analysis since the extraction solvent does not need to be evaporated.

Conclusions

The newly developed supramolecular solvent extraction method based on micelles of 1-decanol dispersed in THF/water continuous phase is a practical and reliable method to separate and preconcentrate five pesticides from environmental water, cereal flour samples before its HPLC-DAD determination. The method supplies limits of quantification that permit the screening and occurrence studies of pesticides in water and cereal flours according to Brazilian and other internationals environmental and public health agencies. The method was successfully used for the determination of pesticides in real samples with acceptable

recoveries ranging from 81 to 111%. Furthermore, this method does not need clean-up and it consumes very low volumes of organic solvents (400 μL of THF), which demonstrates the environmentally friendly behavior of this method, attested by AGREE tool.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Luciane Effting was responsible for conceptualization, methodology, validation, formal analysis, investigation, writing original draft; Letícia M. Effting for conceptualization, investigation, data curation; César Ricardo T. Tarley for supervision, resources, funding acquisition, writing original draft, project administration.

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