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Dispersive liquid–liquid microextraction combined with microvolume spectrophotometry to turn green the 5530 APHA standard method for determining phenols in water and wastewater

Isela Lavilla *, Sandra Gil, Marta Costas, Carlos Bendicho

Departamento de Química Analítica y Alimentaria, Área de Química Analítica, Facultad de Química, Universidad de Vigo, Campus As Lagoas-Marcosende s/n, 36310 Vigo, Spain.

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ABSTRACT

In this work, a new method based on the combination of dispersive liquid–liquid microextraction (DLLME) with microvolume spectrophotometry has been developed as a greener and miniaturized alternative to the 5530 APHA standard method for determining phenols in water and wastewater. The method relies on the oxidative coupling of phenols with 4-aminoantipyrine (4-AAP). In order to preconcentrate the dye formed, the classical liquid–liquid extraction used in the 5530 APHA method (involving 500 mL of sample and 50 mL of trichloromethane) has been replaced by DLLME (with 5 mL of sample, 50 μL of trichloromethane and 200 μL of acetonitrile). After optimization, the method yielded limits of detection and quantification (0.8 and 2.5 μg L^{-1}, respectively) that were comparable with those obtained by the 5530 APHA standard method. Repeatability, expressed as relative standard deviation, was 5.2% ($N=6$), and the enrichment factor (EF) was 700. The proposed method was applied to the determination of phenols in different water samples and a wastewater with recoveries in the range 90–99%. The greenness profile was established in accordance with the suggestions made by the NEMI (National Environmental Methods Index). The absence of PBT (persistent bioaccumulative and toxic chemicals) and corrosive reagents and a drastic reduction of generated wastes can be emphasized.

1. Introduction

Nowadays, there is an increasing demand for green analytical methods with the aim of monitoring organic pollutants such as phenols. These compounds are typically found in domestic and industrial wastewaters, natural waters and potable water supplies. Several phenols are included in the list of priority pollutants by the US Environmental Protection Agency (US EPA) and they are also classified as priority contaminants by the European Union [1,2].

The presence of phenols in the aquatic environment results from both natural and anthropogenic processes. Phenols have numerous uses in industries as those of steel, petroleum, plastic or pharmaceutical. The decomposition of organic matter or the synthesis by fungi and plants are the main natural processes of phenol formation [3]. Phenols are considered extremely hazardous substances for mammals, fish and other aquatic life, and therefore, their levels in waters have been regulated [4].

In order to save time and costs, phenols are usually monitored as total content instead of individual species. Though many analytical methods have been developed for this purpose [5–8], the 5530 American Public Health Association (APHA) standard method continues being the most used in water and wastewater [9]. This method is based on the oxidative coupling of phenols with 4-aminoantipyrine (4-AAP) in the presence of an oxidant to form an antipyrine dye, whose absorbance is measured. For enhancing sensitivity, this substance is extracted from the aqueous solution with trichloromethane. This method is useful to determine phenol, *orto*- and *meta*-substituted phenols. *Para*-phenols where the substitution is a nitro, nitroso benzoyl, alkyl, aryl or aldehyde group do not react with 4-AAP. The main disadvantage of the standard method from the point of view of green analytical chemistry is the use of large volumes of reagents (particularly a hazardous solvent as trichloromethane) and samples.

In order to diminish or eliminate this solvent, several strategies have been developed, e.g., on-line preconcentration of the reaction product by sorbent extraction involving a C_{18}-modified silica microcolumn [10], application of solid-phase spectrophotometry (SPS) combined with an anion-exchange resin [11,12] or removal of the extraction step using a micro-pumped multicommutation system [8]. In comparison with the standard method, these procedures are greener and more sensitive but they cannot be easily adapted for routine analysis.

Miniaturization of sample preparation, especially through liquid phase microextraction (LPME) approaches, constitutes

* Corresponding author. Tel.: +34 986 812291; fax: +34 986 812556.
E-mail address: isela@uvigo.es (I. Lavilla).

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a simple alternative that allows saving of solvents and samples with minimum waste generation [13]. Miniaturization of the classical liquid–liquid extraction (LLE) can be directly performed through the so-called dispersive liquid–liquid microextraction (DLLME) technique [14]. In DLLME, an appropriate mixture of the extraction solvent (with high density) and the disperser solvent (with high miscibility in both extractant and aqueous phase) is injected into the aqueous sample producing a cloudy solution that allows an intimate contact between aqueous and organic phases.

DLLME has been recently applied to the determination of individual phenols in combination with different separation techniques such as high performance liquid chromatography (HPLC) [15,16], gas chromatography (GC) [17] and capillary electrophoresis (CE) [18]. To the best of our knowledge, DLLME has not been used in direct combination with UV–vis spectrophotometry for the determination of phenols.

The miniaturization inherent with DLLME makes it necessary the application of microsample detectors in order to avoid sample dilution. LPME in combination with microvolume spectrophotometry has been recently used for the determination of different analytes as iodide [19], acid labile sulfide [20], thiols, residual free chlorine and chlorine dioxide, ammonia and total iodine [21], trimethylamine-nitrogen [22], chloride [23], iodate [24] and nitrite [25].

In this work, the combination of DLLME with microvolume UV–vis spectrophotometry is proposed in order to turn green the 5530 APHA standard method for determining phenolic compounds in water and wastewater. Variables affecting DLLME were optimised for maximum extraction efficiency of phenols. The proposed methodology was applied to the determination of phenols in different water and wastewater samples.

2. Experimental

2.1. Instrumentation

A Nanodrop® model ND-1000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA) was used for phenol determination in a drop of organic phase (3 μL) after DLLME. Absorption measurements were carried out at 460 nm. A Sigma 2–16 Versatile centrifuge (Montreal Biotechnologies Inc., Dorval, Canada) was used to speed up phase separation. A 10 μL microsyringe (Hamilton model 1710N CTC, Reno, Germany) was used to collect the organic phase. A pH meter Crison model Basic 20 (Alella, Spain) was used for adjusting the pH of samples and standards. High-purity deionised water was obtained from a PETLAB ultrapure water production system (Peter Taboada, Vigo, Spain).

2.2. Reagents and samples

All chemicals were of analytical reagent grade and solutions were prepared with ultrapure water. A stock standard solution of phenol (1000 mg L^{-1}) was obtained dissolving phenol reagent (Merck, Darmstadt, Germany) in water. Working standard solutions were prepared daily by suitable dilution of the stock solution. 4-aminoantipyrine (Sigma–Aldrich, Steinheim, Germany), potassium peroxodisulfate (Merck), potassium hexacyanoferrate (III) (Merck), hydrogen peroxide (Merck), ammonium hydroxide (Sigma–Aldrich) and potassium hydrogen phosphate (Panreac, Barcelona, Spain) were used with derivatization purposes. Dichloromethane (Panreac), trichloromethane (Panreac), toluene (Prolabo, Fontenay-sous-Bois, France), isooctane (Merck, Darmstadt, Germany), xylene (Prolabo) and heptane (Prolabo) were attempted as potential organic extractant phases. Two imidazolium based ionic liquids (ILs) (Merck) were also tried as extractants, 1-butyl-3-methylimidazoliumhexafluorophosphate [C_4MIM][PF_6] and 1-hexyl-3-methylimidazoliumhexafluorophosphate [C_6MIM][PF_6], both without and with sodium dodecyl sulfate (SDS, Fluka, Steinheim, Germany). Methanol (Prolabo), ethanol (Prolabo), acetone (Prolabo), acetonitrile (Prolabo), pyridine (Merck) and *N*,*N*′-dimethylformamide (Merck) were tested as disperser solvents.

Different types of waters (tap, mineral, spring, river and wastewater) were analyzed. The tap water was sampled in the laboratory and the spring water was collected in a natural spring close to the university. A commercial mineral water was obtained from the supermarket and the river water was sampled in the Tea river (Galicia, Spain). The wastewater sample was collected from a municipal wastewater treatment plant. Samples were pretreated according to the procedure 5530 B recommended by the APHA standard method [9].

2.3. DLLME procedure

A 5 mL volume of sample buffered to pH 9.5 with 150 μL of a 0.5 M hydrogen phosphate/ammonia solution was mixed with 50 μL of 1% w/v 4-aminoantipyrine (4-AAP) and 50 μL of 1.5% w/v potassium peroxodisulfate into a 15 mL polyethylene tube and left for 10 min in a water bath (27 °C) so that the colour is allowed to develop. Then, a 250 μL volume of a mixture 1:4 of trichloromethane and acetonitrile was added. The tube was centrifuged for 5 min at 3500 rpm. Finally, a 3 μL drop of the sedimented organic phase was taken with a 10 μL microsyringe and placed in the drop-supporting surface of the microvolume UV–vis spectrophotometer for absorbance measurement. Blanks were treated in the same way.

3. Results and discussion

In order to find the appropriate conditions for DLLME, experimental parameters such as nature and volume of extractant and disperser agent, concentration of 4-AAP and oxidant, reaction time and pH were studied using a phenol standard solution.

Hexacyanoferrate (III) is the oxidizing agent used in the 5530 APHA standard method. However, this reagent showed high blank values, due to absorption of hexacyanoferrate (III) ions and different products of the oxidation of 4-AAP [5]. Then, preliminary experiments were carried out with other oxidants as potassium peroxodisulfate or hydrogen peroxide. Potassium peroxodisulfate provided low blanks and hence, it was chosen for further experiments.

3.1. Nature of the extraction solvent

Greener methods can be achieved using less volume and/or less hazardous solvents. In this sense, trichloromethane, used as extractant in the 5530 APHA standard method, is considered an undesirable solvent regarding safety, environmental and regulatory considerations [26]. However, the polarity of the reaction products to be extracted such as *N*-antipyryl-*p*-benzoquinoneimine formed in the aqueous medium from phenol (pKa 7.17 ± 0.20) [27], makes it difficult to find a suitable alternative among the traditional solvents without impairing the analytical performance.

At first, an alternative to trichloromethane was searched among the traditional solvents. In this sense, Alfonsi et al. [28] proposed dichloromethane as a greener solvent in order to replace trichloromethane as extractant. In addition, dichloromethane can be used in DLLME because it has higher density than water, it forms cloudy

solutions and it has low solubility in water [29]. Nevertheless, when dichloromethane was tried in this work, no extraction of antipyrine dye occurred. Therefore, other traditional organic solvents used in DLLME and considered as usable in the Pfizer solvent selection guide for medical chemistry were tried (heptane, toluene, isooctane and xylene) [26,28]. Poor results were obtained too.

Ionic liquids (ILs) can be considered as interesting extraction agents that improve the greenness profile of analytical methodologies. In special, imidazolium based ILs are useful for liquid microextraction processes [13]. These ILs can be used along with surfactants such as SDS in order to improve their capacity of extraction [30].

The ILs 1-butyl-3-methylimidazoliumhexafluorophosphate [C_4MIM][PF_6] and 1-hexyl-3-methylimidazoliumhexafluorophosphate [C_6MIM][PF_6] were tried in this work as extractants with and without sodium dodecyl sulfate (100 mM SDS). Though extraction occurs, a considerable worsening of sensitivity (four times in comparison with trichloromethane) was observed. Owing to the high solubility of ILs in water, an increased volume of IL is required in comparison with traditional non-soluble extractants (in this case, a minimum volume of IL of 100–150 μL must be used in comparison with 25–50 μL of trichloromethane). These high volumes of IL cause, in turn, blank values to increase.

Then, bearing in mind that the use of green methodologies must not compromise the required analytical characteristics [31] and given that a drastic reduction of extractant volume occurs in DLLME, trichloromethane was finally selected as extractant.

3.2. Nature of the disperser solvent

A disperser solvent miscible with both, the extraction solvent and the aqueous sample was used for enhancing the extraction kinetics [13]. Several organic solvents considered as preferred, usable or undesirable by Alfonsi et al. [28] were tried as disperser agents: methanol, ethanol and acetone (preferred), acetonitrile (usable), pyridine and *N*,*N*′-dimethylformamide (undiserable).

Results are shown in Fig. 1. *N*,*N*′-dimethylformamide and acetonitrile provide an improved absorbance as compared to the absence of disperser solvent. Acetonitrile was finally chosen as disperser agent due to the better greenness profile of this solvent in comparison with *N*,*N*′-dimethylformamide [28].

3.3. Effect of the volume and the ratio of the extractant/disperser mixture

The effect of the total volume of trichloromethane/acetonitrile mixture on the analytical signal was studied in the range 150–400 μL.

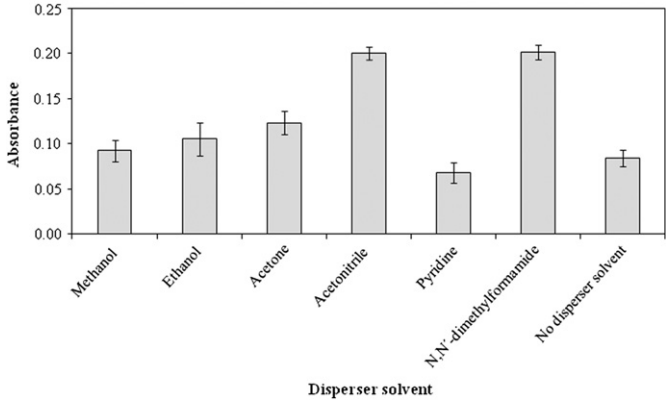

Fig. 1. Effect of different disperser solvents in the DLLME procedure using a mixture of 200 μL of dispersant solvent and 50 μL of trichloromethane as extractant solvent.

Different volume ratios of this mixture were also investigated (i.e., 1:1, 1:2, 1:3, 1:4 and 1:5). Fig. 2A and B show the obtained results. As was expected, higher preconcentration factors were obtained with small volumes of the mixture and trichloromethane/acetonitrile ratio of 1:5 and 1:4. The use of small extractant volumes allows achieving large extraction efficiencies and, in turn, high sensitivity [32]. The ratios 1:5 and 1:4 showed the highest absorbance, but with the ratio 1:5, the volume of sedimented phase after centrifugation was very small, which makes it difficult its withdrawal with the microsyringe prior to analysis. This results in less reproducible results. Mixture volumes between 200 and 250 μL showed the highest absorbance. For smaller volumes, e.g., 150 μL of mixture, the volume of the sedimented phase was also very small. Finally, a 250 μL total volume of the mixture extractant/disperser agent 1:4 was chosen since it provided high extraction efficiency and more reproducible results.

3.4. Reaction conditions

In order to obtain reproducible results, a strict control of pH and concentration of reagents is essential [2]. The effect of pH, adjusted with hydrogen phosphate/ammonia solutions, was studied in the range 8–11. Fig. 3A shows the pH effect on absorbance. A maximum signal is observed in the pH range of 9–10. The maximum stability of colour occurs in the pH range 9.4–10.2 [33], a pH 9.5 being considered as optimum for further experiments.

The effect of the concentration of 4-AAP was also studied. As can be observed in Fig. 3B, an improvement in the signal was obtained when the concentration of 4-AAP in the sample was in the range 0.008–0.010% w/v. A concentration of 0.009% w/v was selected.

An excess of oxidizing agent is undesirable because it can decolourize the dye formed but, on the other hand, low concentrations of oxidant can be insufficient [5,7]. The effect of potassium peroxodisulfate concentration can be seen in Fig. 3B. A concentration of 0.014% w/v potassium peroxodisulfate was considered as optimum for colour development.

When hexacyanoferrate (III) is used as oxidant at room temperature the colour is developed within 15 min [9,11,12]. However, when other oxidants are used, longer times can be required [5]. Fig. 3C shows an increase in the analytical signal up

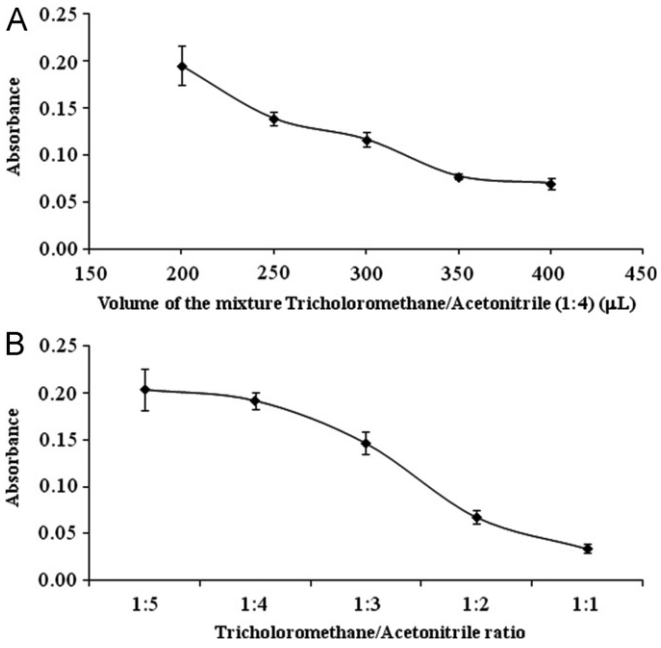

Fig. 2. Effect of the volume (A) and the ratio (B) of the mixture extractant/disperser (trichloromethane/acetonitrile).

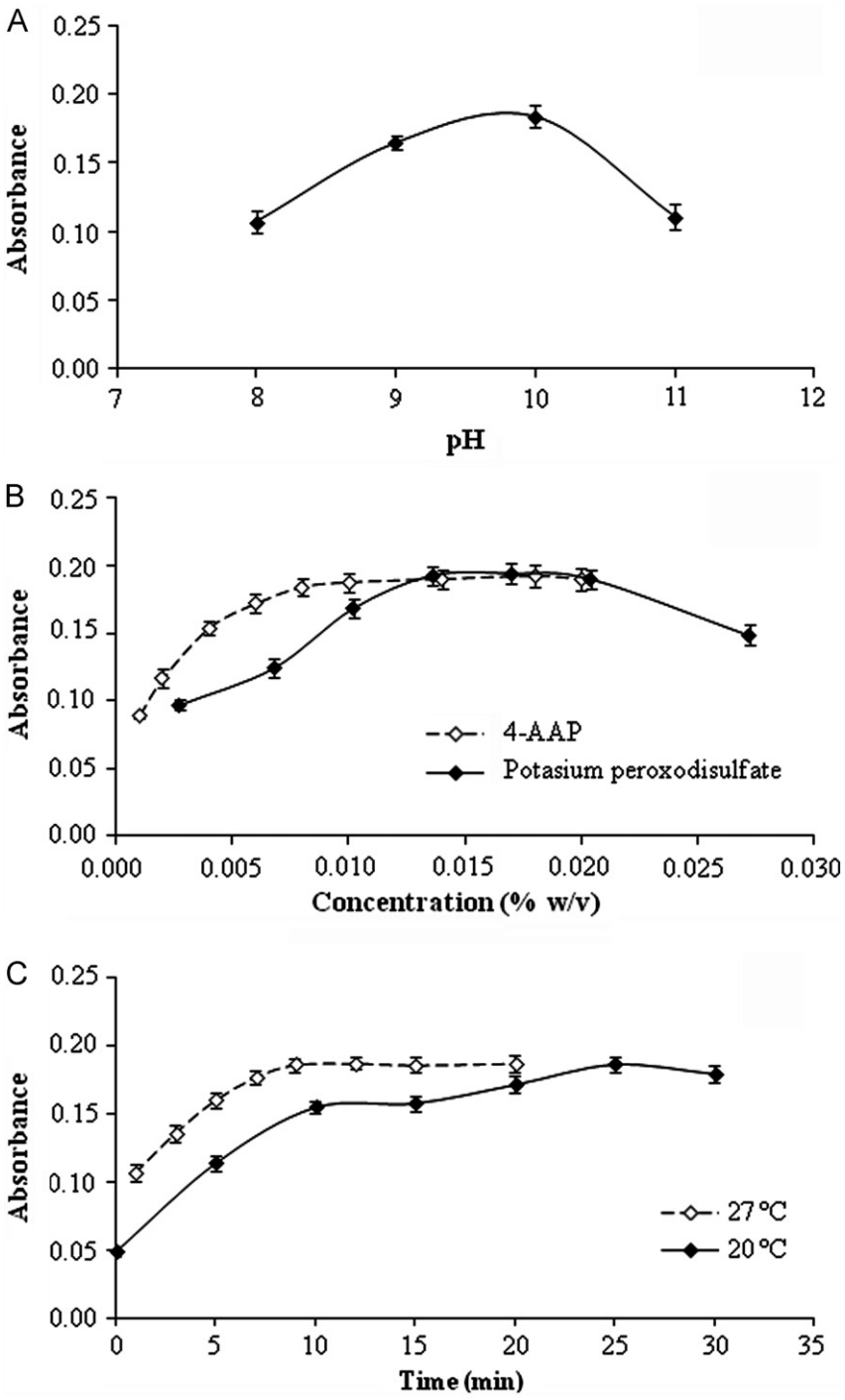

Fig. 3. Study of variables that affect to derivatization reaction: (A) effect of the pH; (B) effect of the 4-AAP and peroxo disulfate concentration; (C) effect of the reaction time with and without temperature bath.

to 25 min working at room temperature (20 °C) with peroxodisulfate as oxidant. When a water bath at 27 °C was used, this increase was observed within 10 min. Additional experiments were carried out up to 45 °C. For temperatures higher than 30 °C, no separation of phases was observed. By working at 27 °C, microextraction can be performed without a previous cooling of the sample.

3.5. Analytical characteristics and sample analysis

Under optimal conditions, linearity, repeatability and limits of detection (LOD) and quantification (LOQ) were obtained. LOD and LOQ were calculated following the 3σ and 10σ criteria, respectively, for a sample volume of 5 mL (Table 1). The calibration was linear from the LOQ up to 150 $\mu g\,L^{-1}$. Repeatability of the method, expressed as relative standard deviation (RSD), was 5.2% ($N=6$). An enrichment factor (EF) of 700 was obtained.

A comparison of the proposed method and the 5530 American Public Health Association (APHA) standard method is shown in Table 1. As can be seen, the decrease in the reagent and sample volumes achieved with the proposed method is remarkable. Analytical sensitivity is similar in both procedures provided that 10-cm cells and 500 mL of sample are used in the APHA standard method for determining phenols in water and wastewater.

Table 1
Comparison of the proposed method and the 5530 American Public Health Association (APHA) standard method for the determination of phenols in water and wastewater.

Parameter	DLLME method	5530 APHA standard method
LOD ($\mu g\ L^{-1}$)	0.8	1 (10-cm cell and 500 mL of sample)
LOQ ($\mu g\ L^{-1}$)	2.5	3.3 (10-cm cell and 500 mL of sample)
Sample volume:	5 mL	500 mL
Reagent concentration and volume:		
Trichloromethane	0.050 mL	25 or 50 (1–5 or 10-cm cell, respectively)
Acetonitrile	0.200 mL	–
Phosphate ammonium buffer	0.5 M; 0.150 mL	0.5 M; 22 mL
4-aminoantipyrine	1% w/v; 0.050 mL	2% w/v; 3 mL
Potassium ferrocyanide	–	8% w/v; 3 mL
Potassium hexacyanoferrate(III)	1.5% w/v; 0.050 mL	–
Sample preparation time:	10 min reaction+5 min centrifugation	15 min reaction+two times extractions in separatory funnels+filtration of the extracts

Table 2
Some selected DLLME based methods applied to phenol determination in waters for comparison with the proposed DLLME-microvolume UV–vis spectrophotometric method.

Method	LOD ($\mu g\ L^{-1}$)	Lineal range ($\mu g\ L^{-1}$)	RSD (%)	Extraction solvent	Disperser solvent	Sample	EF	Sample volume (mL)	Refs.
DLLME-HPLC-VWD	0.68–10	4–400	1.9–4.8	50 μL of 1-butyl-3-methylimidazolium hexafluorophosphate	-	Tap, river water and wastewater	n.r.	1.5	[15]
DLLME-HPLC-DAD	0.01–1.3	0.1–500	2.6–16.6	165 μL of carbon disulfide	2.50 mL of acetone	Industrial wastewater	30–373	5	[16]
DLLME-GC-ECD	0.01–2.0	0.02–400	0.6–4.7	10 μL of chlorobenzene	500 μl of acetone	Well, tap and river water	287–906	5	[17]
DLLME-HPLC-DAD	7–29	0.05–100	11.2–13.9	50 μL of tri-*n*-butylphosphate	0.5 mL of methanol	Tap, lake, fishpond waters, sewage and industrial wastewaters	35.4–55.3	3.7	[34]
SA-DLLME-HPLC-UV	0.1	0.2–200	4.7–6.9	35 μL of 1-octanol and 0.09 mmol L^{-1} CTAB	–	Tap water, mineral waters and seawater	187–353	11	[35]

- Without disperser solvent
n.r.: Not reported
VWD, variable wavelength detection; DAD, diode array detection; ECD, electron capture detector; SA, surfactant assisted; CTAB, cethyltrimethyl ammonium bromide

Table 3
Determination of phenols in waters and wastewater.

Sample	Phenol added($\mu g L^{-1}$)	Phenol found($\mu g L^{-1}$)	Recovery(%)
Tap water	–	< LOQ	–
	50	49.7 ± 2.8	99 ± 6
Mineral water	–	< LOQ	–
	50	49.4 ± 3.6	99 ± 7
Spring water	–	< LOQ	–
	50	48.7 ± 4.1	98 ± 8
River water	–	< LOQ	–
	50	44.9 ± 2.6	90 ± 6
Wastewater	–	47.1 ± 3.9	–
	50	93.3 ± 5.0	90 ± 5

The analytical characteristics of the proposed method were compared with those provided by other DLLME methods described in the literature for the determination of phenols in water and wastewater samples (Table 2). The obtained LOD is comparable to that provided by other methodologies. It is remarkable that the proposed method, together with the DLLME-GC-ECD methodology, allows achieving the highest EFs.

Three drinking waters (tap water, mineral water and spring water), one river water and one wastewater were analyzed using the developed methodology. The concentration of phenols was below the quantification limit in all samples under study, except in the wastewater. Samples were spiked with 50 $\mu g\ L^{-1}$ of phenol and then recovery values were calculated. The obtained recoveries were between 90–99% in all cases (Table 3).

Fig. 4. Greenness profile of the proposed methodology in accordance with the suggestions made by NEMI (National Environmental Methods Index).

3.6. Greenness profile of the proposed methodology

The greenness profile was established in concordance with the suggestions of the NEMI (National Environmental Methods Index) [31,36]. The presence of PBT (persistent bioaccumulative and toxic chemicals), hazardous and corrosive reagents as well as the generated waste were assessed. Fig. 4 shows the pictogram suggested by the NEMI for the proposed methodology. In this case, the amount of waste generated is < 50 g (green quadrant), the pH is no corrosive (green quadrant), PBTs were not used (green quadrant) and only the hazardous quadrant is white since acetonitrile, ammonia and trichloromethane are listed on the TRI (Toxic Release Inventory) program [37].

4. Conclusions

In this work, the environmental impact of the 5530 American Public Health Association (APHA) standard method has been

drastically reduced by decreasing the volumes of reagents and sample. Miniaturization of sample treatment and measurements steps using dispersive liquid–liquid microextraction combined with microvolume UV–vis spectrophotometry allows turn green the standard method without compromising the analytical characteristics. The use of less hazardous substances as extractants (e.g., ILs) was also explored but analytical characteristics worsened. It is remarkable to observe that the LOD of the 5530 APHA standard method is comparable to that obtained with DLLME only when 500 mL of sample and special conditions for measurement (10-cm cell) are used. The proposed method is well suited to the routine laboratory in contrast with other attempts to turn green the determination of phenols.

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