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Parallel artificial liquid membrane extraction of organophosphorus nerve agent degradation products from environmental samples

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A b s t r a c t:

An emerging miniaturized high-throughput microextraction technique named Parallel artificial liquid membrane extraction (PALME) was, for the first time, investigated for the extraction of polar alkyl methylphosphonic acids (AMPAs) that are the degradation products of organophosphorus nerve agents. The effect of the key-parameters of the extraction method (nature of the membrane, of the extraction solvent, of the pH values of both donor and acceptor phases, agitation speed, extraction time, temperature and ionic strength) on the extraction recoveries was studied in spiked pure water samples. This led to extraction recoveries in the range of 25-102% for the 5 targeted analytes from water with enrichment factors in the range of 4.50-42.75.

The developed PALME-LC-MS/MS method was first evaluated with spiked pure water. LOQs (S/N≥10) were in the range of 0.009–1.141 ng mL^{-1}, linearity above 0.9973 for all the AMPAs and with RSD values below 11%. This method was then applied on simulated waste water, river water and aqueous soil extracts. The achieved LOQs were in the range of 0.011-1.210, 0.013-1.196 and 0.016-6.810 ng mL^{-1}, respectively. A detailed comparison of the performances of this PALME method with those of a previously developed hollow fiber liquid-phase microextraction methods already applied to AMPAs was done thus allowing to demonstrate the easy transfer of methods from HF-LPME to PALME. Moreover, the high-throughput potential of PALME was revealed since 192 samples were processed in parallel during 120 minutes (37.5 sec/sample).

Key words: Parallel artificial liquid membrane extraction; alkyl methylphosphonic acids; chemical warfare agent degradation products; LC-MS/MS analysis; environmental samples

Abbreviations:

AMPAs: alkyl methylphosphonic acids; CMPA: cyclohexyl methylphosphonic acid; EF: enrichment factor; HBA: hydrogen Bond acceptor; HBD: hydrogen Bond Donor; iPrMPA: isopropyl methylphosphonic acid; EMPA: ethyl methylphosphonic acid; EPA: ethylphosphonic acid; HF-LPME : hollow fiber liquid-phase microextraction; iBMPA: isobutyl methylphosphonic acid; LPME: liquid-phase microextraction; MPA: methylphosphonic acid; OPCW: Organization for the Prohibition of Chemical Weapons; PALME: parallel artificial liquid membrane extraction; PGC : porous graphitic carbon; PMPA: pinacolyl methylphosphonic acid; PP: polypropylene; PPA propylphosphonic acid; PVDF polyolyvinylidene fluoride; SCX : strong cation exchange; SLM : supported liquid membrane.

1. Introduction

Since World War I, the extensive use of chemical weapons causing great damage led to the establishment of the Chemical Weapons Convention to prohibit the use, production and stockpiling of chemical warfare agents. However, chemical warfare agents are difficult to detect because they degrade in the environment within a few hours to several days [1]. This study focuses on the analysis of five alkyl methylphosphonic acids (AMPAs), which are environmental markers of the main nerve agents [2]. Their analysis remains a challenge due to their highly polar character (log P values between -0.9 and 1.4) and acidic property with pKa values around 2 (see Table S1 for more details). Their presence at trace level in complex samples implies the need for a powerful and effective sample treatment step in order to extract and concentrate them before chromatographic analysis.

Among analytical techniques that have been used for AMPAs analysis in environmental samples, liquid chromatography in combination with mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) constitute the two most prominent ones [3]. For the GC analysis of AMPAs, a derivatization step is mandatory to convert these non-volatile analytes into GC-amenable compounds. However this chemical modification may introduce artefacts and/or could be a source of errors, especially false positive responses [4]. Thus, LC-MS proved to be an attractive alternative, as the analytes of interest can be directly analyzed in their intact form without the need for additional sample handling and derivatization steps. Moreover, the combination of LC with tandem mass spectrometry (MS/MS) offers the greatest level of specificity and sensitivity for their analysis [5].

Considering the LC separation, the HILIC mode [5, 6], mixed-mode [7] and porous graphitic carbon systems [8] were used but reversed-phase liquid chromatography on C18 silica was the method of choice for their separation [9–11]. However, their presence at low concentration in a matrix with a high level of interfering compounds constitutes a real challenge. Therefore, a sample treatment method is crucial to achieve a selective enrichment of the target analytes and to ensure an efficient cleanup of the sample prior to the LC–MS analysis [6].

To replace the conventional extraction techniques like Solid Phase Extraction (SPE) [12-17], miniaturized sample preparation known as liquid-phase microextraction (LPME) has emerged. It consists of the use of the porous polypropylene hollow fiber based liquid-phase microextraction (HF-LPME) [18]. Even if low extraction recoveries (lower than 30%) were obtained for polar compounds because of their low affinity for organic solvents, as target compounds are extracted into a very small volume of acceptor phase, a high enrichment factors (EF's) could be obtained [19]. For this reason, experts of the Scientific Advisory Board of the organization for the prohibition of chemical warfare considered that HF-LPME is a very promising technique and can be recommended as an operational procedure for on-site and off-site analysis [20]. Two-phase HF-LPME was applied for the extraction of AMPAs in combination with different derivatization agents such as propyl bromide [8], N-tert-butyldimethylsilyl-N-methyltrifluoroacetamid (MTBSTFA) [9] or 3,5 trifluoromethyl benzene diazomethane [10]. Nevertheless, while removing the constraints linked to the derivation step, higher EFs were obtained with the three-phase HFLPME. Indeed, Tak *et al.* reported EFs of AMPAs in the range of 11-135 in pure water [8]. By introducing heating to the same set-up and adjusting some other operational parameters, Desoubries *et al.* applied it on real environmental and biological samples [14] and the obtained EFs and LOQs in river water were in the range of 15-220 and 0.013–5.3 ng mL^{-1} respectively. However, this technique is time consuming because it is performed sequentially for each sample (50-60 min per 3-6 mL of sample) and it demands highly trained analysts. Thus, trends are for the development of high-throughput sample processing techniques that (i) meet the fundamental

principles of green chemistry, (ii) are easy to implement, that (iii) allow the processing of hundreds or even thousands of samples per day in a reliable and reproducible manner and (iv) offer a practical and inexpensive alternative to other complex and laborious traditional techniques [21]. The group of S. Pedersen-Bjergaard has thus adapted in 2013 the HF-LPME concept in 96-well standardized device named parallel artificial liquid membrane extraction (PALME) [22]. Basically, the target compounds are extracted from an aqueous sample through a thin film of organic solvent, which is loaded as a supported liquid membrane (SLM) into the pores of a flat membrane, and end up in a solution located in the wells of the acceptor plate. Like HF-LPME, PALME is based on a passive and one-way extraction principle but with a different geometry as illustrated in Fig. S1A. The PALME has been used for the treatment of biological samples like plasma [23] or whole blood [24] and recently for a food matrix (wine) [25]. Its application to nonpolar compounds was performed at many occasions: whether for basic [24, 26, 28–32] or acidic [27, 30, 31, 33] products. Such as HF-LPME, PALME technique is generally recommended for the extraction of moderately to non-polar compounds (log P> 1.5-2) [21, 34]. When the analytes are polar, their extraction becomes problematic without resorting to carriers to facilitate their transfer across the SLM to the acceptor phase. Therefore, the formation of a temporary ion-pair complex between the hydrophilic analyte and 2-di(ethylhexyl) phosphate, that was added in the SLM to form ion-pairs, was proposed for the extraction of polar basic drugs [23]. Another approach consists on the optimization of the SLM using a combinations of different solvents [19].

The purpose of this work was thus to develop a simple, robust and practical high-throughput LPME method helping responding mobile and off-site laboratories to process the large number of samples that could be associated to large scale nerve agent event.

Therefore, a PALME method was developed for the extraction of five AMPAs from environmental samples. The studied analytes were pinacolyl methylphosphonic acid (PMPA), isopropyl methylphosphonic acid (iPrMPA), cyclohexyl methylphosphonic acid (CMPA), ethyl methylphosphonic acid (EMPA), isobutyl methylphosphonic acid, (iBMPA), which are primary hydrolysis products of the nerve agents soman, sarin, cyclohexyl-sarin, VX, and Russian-VX respectively. The effect of various extraction parameters commonly studied in PALME like pH of the donor and acceptor phases, agitation speed and time of extraction on recoveries and on enrichment factors was studied. The selection of new type of donor plates (deep well) with a larger volume (2.2 mL) than the ones commonly used in PALME (0.5 mL), the membrane material and the integration for the first time of new operational parameters like salt concentration in the sample and control of the temperature of the extraction were also examined. The best extraction conditions were applied to the extraction of AMPAs from environmental samples (surface waters and aqueous soil extracts) to evaluate the performance of this method in terms of sensitivity, reproducibility but also of matrix effects on recoveries.

2. Experimental

2.1. Chemicals

Soman acid (pinacolyl methylphosphonic acid, PMPA, 97%), VX acid (ethyl methylphosphonic acid, EMPA, 98%), sodium chloride (NaCl), octyl ether, dihexyl ether, hexadecane, dodecanol and 1-octanol were from Sigma–Aldrich (Saint-Quentin-Fallavier, France). Sarin acid (isopropyl methylphosphonic acid, iPrMPA, 99%), cyclohexyl-sarin acid (cyclohexyl methylphosphonic acid, CMPA, 96%), and Russian VX acid (isobutyl methylphosphonic acid, iBMPA, 99%) were synthesized in DGA CBRN Defense (Vert-le-Petit, France). Purified water was obtained from an Alpha-Q purification system (Millipore, Saint-Quentin-en-Yvelines, France, 18.2 MΩ). Acetonitrile (ACN) was from Carlo-Erba (Val-de-Reuil, France). Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Merck (Darmstadt,

Germany) and they were employed to adjust the pH in the aqueous samples and acceptor solutions, respectively. All reagents and solvents used were of analytical grade.

2.2. Standard solutions and samples

Stock standard solutions of each AMPA were prepared in ACN at a concentration of 100 μg mL^{-1} and stored at +4 ◦C until further use. The stock solutions were used for spiking pure water, river water (Seine river, France), simulated waste water (250 μg mL^{-1} of $CaCl_2$, 100 μg mL^{-1} of $MgSO_4$ and 250 μg mL^{-1} of PEG400 in pure water) and aqueous soil extracts to obtain a concentration of 100 ng mL^{-1}. All these solutions were used as sample solutions after adjustment of their pH between 0 and 3 with concentrated HCl. An appropriate amount of NaCl, from 0 to 30% (w/v) depending on the experiments, was added. The pH of the acceptor solutions was adjusted between 11 and 14 using sodium hydroxide (NaOH).

To evaluate the performance of the PALME method in pure water, solutions at 5 concentration levels (0.5, 5, 10, 50 and 100 ng mL^{-1}) of the targeted AMPAs except for EMPA (5, 10, 50 and 100 ng mL^{-1}) were prepared.

The soil sample was collected from Parc de Sceaux (Hauts-de-Seine, France) at 0-5 cm depth. No sieving or any other type of physical treatment was applied on it. The aqueous soil extracts were obtained according to two similar sample preparation methods intended for the exhaustive extraction of iPrMPA, PMPA [33], methylphosphonic acid (MPA), ethylphosphonic acid (EPA) and propylphosphonic acid (PPA) [34]. They extraction method is mainly based on sonication of soil with water and centrifugation steps: a 10 min sonication step followed by a centrifugation at 700G for 10 min was depicted in one case [33] and a 5 min sonication step followed by a centrifugation step at 10 000 rpm (time not mentioned) was depicted in the second one [34]. The main notable difference between these methods lies in the volume of water used for the extraction compared to the amount of the extracted soil. The first method uses a ratio of 1:1 (V/W) while this was fixed at 5:1 (V/W) for the second one. The two types of the prepared aqueous extracts (sonication for 10 min, 700G for 10min) and (sonication for 5min, 4000G for 10min) were spiked with the five targeted AMPAs at a concentration of 100 ng mL^{-1}. These extracts were not filtered by syringe filter since the PALME technique is based on the use of a membrane.

2.3. LC-MS/MS analysis

The AMPAs were analyzed using an LC-MS/MS Agilent 1200 LC system (Agilent Technologies, Les Ulis, France) coupled to an Ultivo triple quadrupole mass spectrometer (Agilent Technologies) controlled by Mass Hunter software (Version 10.0). The separation of AMPAs was accomplished with an Atlantis dC18 column (150 mm × 2.1 mm, 5 μm) by using a gradient from 100% H_2O acidified with 0.1% formic acid (A) to 90% ACN acidified with 0.1% formic acid (B) during 10 min at a flow-rate of 200 μl min^{-1}. Then, this mobile phase composition was kept constant for 1 min before returning to equilibrium. The injection volume was set at 5 μl.

The ionization was carried out with an electrospray interface (ESI) in a negative ion mode. Acquisition was performed in a multiple reaction monitoring (MRM) mode. The parameters of the ESI source were as follows: drying gas (N_2) flow rate, 8.0 L min^{-1}; drying gas temperature, 350 °C; nebulizing gas pressure, 30 psi. The MRM transitions and corresponding parameters are shown in Supporting Information (Table S2).

2.4. Equipment and extraction set-up

PALME was performed using a 96-well plate of polypropylene (PP) with 0.5 mL wells from Nunc (Roskilde, Denmark) or 96-well deep-well collection plates (Millipore, Germany) with 2.2 mL wells as donor plate depending the experiments, and a MAIPNTR10 96-well MultiScreen-IP Filter Plate with

0.45 µm porous, polyvinylidene fluoride (PVDF) membrane (Millipore, Ireland) as an acceptor plate with a maximum working volume of 250 µL. To evaluate the impact of the membrane material, a PP membrane (Accurel PP 1E R/P, Membrana, Wuppertal, Germany) was cut into circular pieces, and they were attached between the donor and acceptor plates. The internal diameter of each well was 6 mm. A lid was used to avoid potential losses of the acceptor solution by evaporation during extraction. No preconditioning of the membranes was required.

The extraction procedure was performed as follows. First, the acidified samples solutions were pipetted into the 96-well donor plate (0.5 mL wells or 2.2 mL wells depending on the experiments). Then, 4 µl of various organic solvents were deposited into each porous membrane in the acceptor plate to form the artificial liquid membrane. Finally, a volume of 50 µl of alkaline acceptor phase (NaOH) was introduced into the 96-well acceptor plate. The latter was sealed with Thermowell™ sealing tape. Finally, the two plates were fixed together by tape. The set-up is illustrated in Fig. S1B.

The whole assembly was agitated and heated on a microplate shaker with 2-place platform (PHMP Grant-Bio, Riga, Latvia) for a given time. After PALME, the acceptor solutions were collected using a micropipette. 40 µl of the recovered solutions were then neutralized by the same volume of HCl solution (1:1, v/v) to be compatible with the developed LC–MS/MS analysis. The recoveries R (%) and EFs were estimated according to the following equation:

$$R = \left(\frac{n_{a,final}}{n_{d,initial}}\right) * 100\% = \frac{C_{a,final}}{C_{d,initial}} * \frac{V_a}{V_d} * 100\% \; and \; EF = R * \frac{V_d}{V_a}$$

Where $n_{d,initial}$ is the initial amount of analyte in the donor phase and $n_{a,final}$ is the final amount of the analyte present in the acceptor phase estimated thanks to the calibration curve established in pure media. $C_{d,i}$ and $C_{a,f}$ are the concentrations of analyte present, respectively, in the donor and acceptor phases. V_d and V_a represent the volumes of donor and acceptor solutions, respectively.

The limits of quantification (LOQs) after sample treatment were calculated for a signal-to-noise ratio (S/N) of 10 using results obtained by applying PALME-LC–MS/MS to the five samples (PW: pure water; RW: river water; SWW: simulated waste water, ASE1: aqueous soil extract 1:1 (V/W) and ASE2: aqueous soil extract 5:1 (V/W)) spiked at low concentrations ($[PMPA]_{PW,\ RW\ and\ SWW}$ = 0.05 ng mL^{-1} and $[PMPA]_{ASE1\ and\ ASE2}$ = 0.1 ng mL^{-1}; $[CMPA]_{PW,\ RW\ and\ SWW}$ = 0.05 ng mL^{-1} and $[CMPA]_{ASE1\ and\ ASE2}$ = 0.1 ng mL^{-1}; $[iBMPA]_{PW,\ RW\ and\ SWW}$ = 0.1 ng mL^{-1} and $[iBMPA]_{ASE1\ and\ ASE2}$ = 0.2 ng mL^{-1}; $[iPrMPA]_{PW,\ RW\ and\ SWW}$ = 0.1 ng mL^{-1} and $[iPrMPA]_{ASE1\ and\ ASE2}$ = 1 ng mL^{-1}; $[EMPA]_{PW,\ RW\ and\ SWW}$= 5 ng mL^{-1} and $[EMPA]_{ASE2}$ = 10 ng mL^{-1}).

3. Results and discussion

3.1. Optimization of PALME parameters

The first series of experiments consisted of studying the key-parameters affecting the extraction efficiency such as the nature of the extraction solvent loaded in the membrane, the chemical composition of this membrane, the pH values of the donor and acceptor phases, the sample ionic strength, the agitation speed, the temperature and the extraction time. Moreover, to reach high EF's, the volume donor phase, *i.e.* of sample, was also optimized by selecting a donor plate endowed with deeper wells than conventional plate used in the previous works. In order to limit the consumption of non-commercially available compounds (iPrMPA, CMPA and iBMPA), and as PMPA was previously used for the optimization of HFLPME [9], the effect of most of these parameters on recoveries were studied by spiking pure water with this compound.

Therefore, first extraction tests were carried out using commercially available 96-well plates with PVDF membrane during 30 min at room temperature (25◦C) with an agitation speed of 600 rpm without any salt addition to the samples. The volumes of the acceptor and donor phases were 350 µL and 50 µL, respectively. All these parameters were drawn up according to the first report published on PALME

[22] while the pH's values of the donor and acceptor phases, *i.e.* 1 and 13, respectively, were close to those reported for the extraction of AMPAs by HF-LPME in triphasic mode, *i.e.* 1 and 14 respectively [8, 9].

3.1.1. Selection of the supported liquid membrane

The selection of a suitable organic solvent to form the SLM is a crucial step for efficient extraction. Thanks to its high affinity with targeted analytes, 1-octanol was already selected for the three-phase HF-LPME of AMPAs [8, 9]. However, it was judged necessary to check its compatibility with the new filter material (PVDF) since it was only applied on a PP material before. In addition to 1-octanol, a selection of four solvents already applied in PALME or in HF-LPME (dihexyl ether, octyl ether, dodecanol and hexadecane) were also examined. Hexadecane and ether-based solvents like dihexyl ether were selected for the PALME extraction of either acidic or basic drugs [24, 30, 31, 33]. Dodecanol was selected for the HF-LPME of pesticides from grape juice instead of octanol and toluene [35].

The extraction recoveries obtained for the five tested solvents are reported in (Fig. S2). Extraction recoveries for dodecanol and hexadecane were 16 and 9% respectively while almost no recovery was obtained using the two ether-based solvents. This could be explained by the fact that the selected ethers act only as hydrogen bond acceptors (HBA) while the AMPAs are also HBA. The highest extraction recovery (44%) was provided by 1-octanol. Thus, it was selected as an extraction solvent for all further experiments.

The obtained results show that polarity (log P) alone could not explain the affinity of the solvent to the studied analytes. The experimental parameters of the Kamlet–Taft solvatochromic relationship which measure separately the hydrogen bond donor (α), hydrogen bond acceptor (HBA: β), and dipolarity/polarizability (π*) properties of solvents as contributing to the overall solvent polarity were used recently to explain this aspect [36]. Since 1-octanol was found to have a relatively high values for α which means it has a high HBD strength and thus will accommodate the HBA groups of AMPA's. In general, 1-octanol has affinity to a huge board of compounds. It was widely used as a solvent in HF-LPME [37] thanks to its ability to act as HBD and as HBA depending the type of the analyte.

3.1.2. Selection of the membrane material

A previous study compared fibers made of PP and PVDF and showed that the fiber with the PVDF material was more suitable for the HF-LPME of flunitrazepam in plasma and urine [38]. Furthermore, the combination of the PVDF material and the 1-octanol revealed the best extraction efficiency for organochlorine pesticides compared to the PP material used in combination of various organic solvents [39]. In return, many studies involving PALME were accomplished by changing the PVDF membrane by PP membrane to avoid the problem of the nonspecific binding of the studied analytes to the PVDF filter membrane but without giving no further information or comparison between these membranes [24, 25, 30, 33]. As the HF-LPME systems for the extraction of AMPAs were built using PP fibers [8, 9], we investigated the effect of replacing the PVDF Immobilon®-P membrane in the acceptor plates by the porous PP membrane on the extraction performance. This was done by studying the behavior of most and the less polar analytes (PMPA and EMPA). The differences between the two membranes are given in (Table S3).

With an extraction time of 30 minutes, the extraction efficiency for both analytes obtained for the PVDF Immobilon®-P were higher than those obtained for PP Accurel PP 1E **(**R/P**)**, as shown in Fig. 1. Indeed, the extraction recoveries obtained for the PVDF membrane were 53 and 6% while they do not exceed 31 and 4% with the PP membrane for PMPA and EMPA respectively. These low extraction recoveries obtained using the PP membrane could be due to (i) kinetics of exchange for PMPA through both membranes or (ii) to the relatively small pore size of this membrane compared to the PVDF

membrane and/or to its chemical nature. Indeed, the impregnation of the PVDF membrane by the solvent was instantaneous which was not the case for the PP membrane as previously reported [40]. Therefore, no optimization of the immersion time in 1-octanol of the PVDF membrane needs to be performed in contrast to what had been done previously in HF-LPME [9]. Moreover, the filter plates with PVDF material are commercially available in contrast of PP material. For all these reasons, the PVDF membrane was selected for the next experiments.

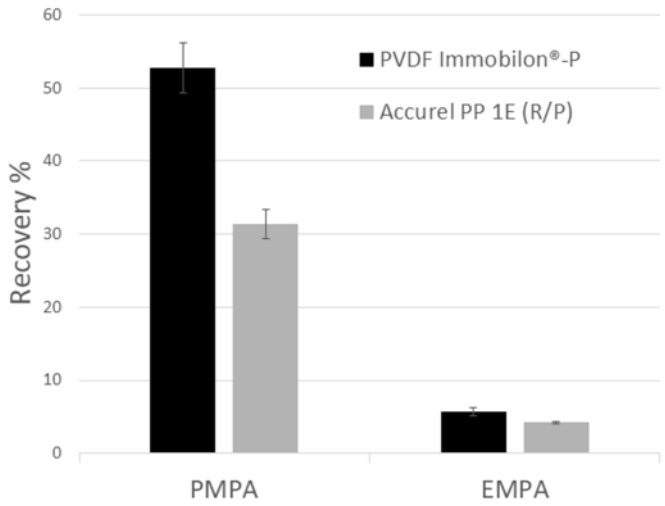

Fig. 1: Effect of the type of the flat membrane on the extraction recovery of 500 ng mL^{-1} of EMPA and 100 ng mL^{-1} of PMPA from pure water by PALME (n = 3). Extraction conditions: donor phase: pure water at pH 1, V_d=350 µL, 0 % NaCl (w/v); acceptor phase: pure water at pH 13, V_a = 50 µL; 4 µL of octanol; extraction time: 30 min; T_{ext} = 25 ◦C; agitation rate: 600 rpm.

3.1.3. Effect of the pH of donor and acceptor phase on recoveries

In this three-phase LPME mode, the extraction process is pH dependent. This involves adjusting the pH of the sample to an acidic pH so that the analytes are not ionized, thus facilitating their transfer through the SLM to the aqueous acceptor phase, which must be at a basic pH to prevent their re-extraction into the organic solvent and thus ensure a mono-directional transfer.

The effect of the pH of both donor and acceptor phases on the extraction recovery of PMPA was studied and the results are reported in the Fig. 2A and Fig. 2B. The best extraction recoveries were achieved with pH values of 0 or 1 for the donor phase. Hence, the pH value of the donor phase was set at 1. Regarding the pH of the acceptor phase, it was found that higher concentrations of NaOH in the acceptor phase provided higher recoveries for PMPA. Theoretically, the transfer of analytes to the acceptor phase should be effective at pH values of the acceptor phase that are higher than the pKa values of the analytes. Thus, a pH value above 5 (pH > pKa + 2) should be more than sufficient. However, it was mentioned earlier that a large pH difference between the donor and acceptor phases is necessary to ensure the extraction of these highly polar compounds [8, 9]. Therefore, pH values of the acceptor phase between 11 and 14 were tested. The latter was found to give the best recoveries for PMPA. Hence it was selected as the optimum pH value for the acceptor phase for the next experiments. It can be noticed that optimized values of the pH of both donor and acceptor phase are exactly the same as those optimized for the HF-LPME of PMPA in a previous studies [8, 9].

3.1.4. Effect of the ionic strength on recoveries

While not studied in previous works on PALME, past HF-LPME studies on the extraction of AMPAs from aqueous samples reported the positive effect of the salt addition on the extraction process (salting out effect) of PMPA [8, 9]. Therefore, the salting out effect was studied by adding NaCl to the donor aqueous phase in the range of 0–30% (w/v). The addition of the salt in the donor phase is thus limited by the solubility of NaCl in water (≈ 33%, at 25 ◦C). The results reported in Fig. 2C show that the extraction efficiency of the PMPA is increasing when increasing the salt concentration. The addition of 30% NaCl (w/v) led to a twofold increase in PMPA extraction recoveries. Thus, the 30% (w/v) salt concentration was chosen as the optimal concentration. However, as PMPA is the compound for which the highest yield is expected (as it is the least polar) and that the yield is close to 100% by adding 30% NaCl, the study of the other key extraction parameters was continued without adding NaCl to the donor phase in order to simulate a compound that is more difficult to extract and to gain a better understanding of the effect of the other parameters still to be studied on the yield.

3.1.5. Effect of the extraction temperature on recoveries

In all previous work on PALME the effect of extraction temperature as a critical parameter has not been taken into account. However high-throughput sample preparation is based on automation with the objective of reducing the susceptibility to errors and variability as much as possible. In fact, the control of the temperature could ensure a better inter-day repeatability of the extraction recoveries. Moreover, the temperature can improve the diffusion coefficients of the analytes and decrease the time required to reach the equilibrium. The effect of the temperature on the PALME efficiency of PMPA was thus investigated by increasing the temperature from 25°C to 60°C. As shown in Fig. 2D, the recoveries first increase with temperature and reach an optimum at 40°C before decreasing slightly. As already observed on different compounds extracted with HF-LPME [41,42,43], this decrease could be due to a decrease of the partition coefficient following the increase of the temperature and/or to the beginning of a loss of organic solvent from the SLM by solubilization in aqueous media and evaporation. Consequently, a temperature of 40 ◦C should be chosen as the optimum extraction temperature. These results confirm those of Desoubries *et al*. [9] who reported an increase and then a decrease in the EF of PMPA as a function of temperature in HF-LPME and determined an optimal extraction temperature of 42°C.

3.1.5. Effect of the agitation speed on recoveries

As with any method based on partition equilibrium, the speed of agitation is an important parameter for improving extraction kinetics. However, it is obvious that the value of agitation speed is also depending on the system used for agitation and of the whole experiment set-up.

Unlike in HF-LPME where the stirring affects only the donor phase, the stirring in PALME promotes the mass transfer of the analytes by convection in both sample donor phase and acceptor phase. Moreover, the system of agitation in PALME is almost standardized therefore 900 rpm was optimized once [22] and fixed as an optimum value for the agitation speed in all the previous works with PALME carried out by the inventor team.

In this study, different agitation speeds ranging from 200 to 1200 rpm were tested to determine their effects on the extraction efficiency of PMPA. As shown in Fig. 2E, extraction recoveries increase when increasing the agitation speed from 200 to 600 rpm. For agitation speeds higher than 600 rpm, the recoveries are constant, thus indicating that, the equilibrium is already reach in 30 minutes. 1000 rpm was selected as an optimum agitation speed. This result is very close to the one used for all the previous PALME studies. This can be explained by the standardization of the technique unlike the HF-LPME

where different types of agitation platforms, size of vials, volumes of samples, immersion length of the HF, size of magnetic stir bars could affect value of the optimal agitation speed.

3.1.6. Effect of the extraction time on recoveries

Like the other microextraction approaches, the mass transfer in PALME is a time dependent process and continuously increased until equilibrium is reached between donor phase, SLM and acceptor phase. It can take from few minutes to several hours to attend an optimal extraction recovery and EF. The results obtained while increasing the extraction time from 30 to 50 min with an agitation speed fixed at 600 rpm showed that the extraction efficiency of the PMPA increased considerably (from 40 to 80%) (Fig. 2F) before reaching a plateau for higher extraction times. Therefore, setting an extraction time of 60 minutes ensures that equilibrium is reached, even more so if a higher stirring speed is set as suggested by the results described in section 3.1.5.

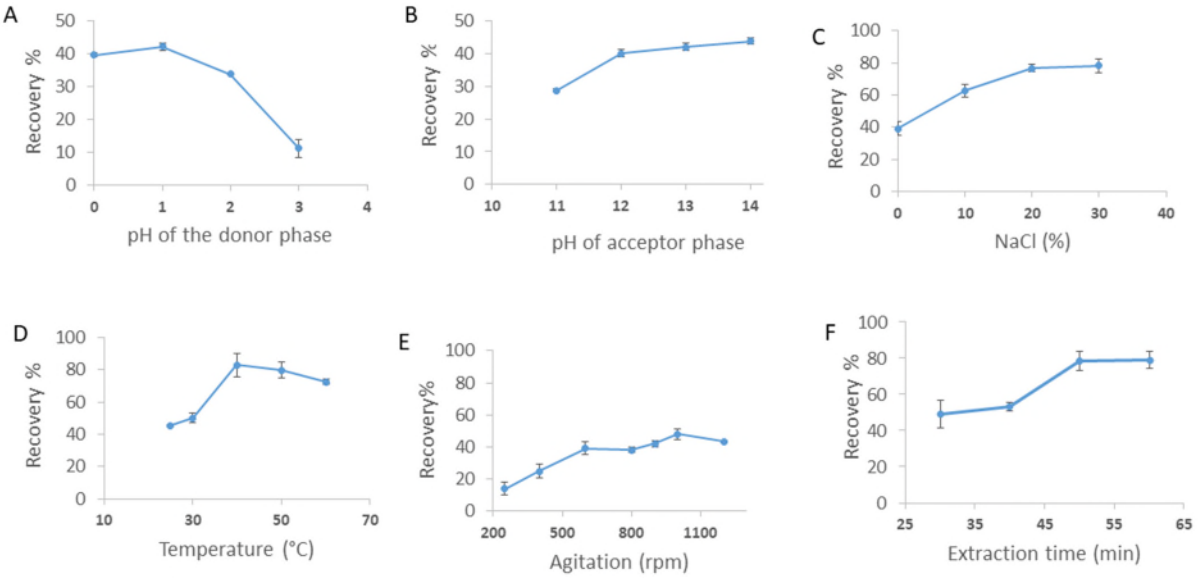

Fig 2: Effect of the pH of the donor phase (A) and of the acceptor phase (B), of the amount of NaCl in the donor phase (C), of the temperature (D), of agitation speed (E) and of the extraction time (F) on the extraction recoveries of PMPA (100 ng mL^{-1}) from pure water by PALME (average ±SD, n=3). Constant parameters from A to F (except when mentioned): 4 µL of 1-octanol in PVDF membrane; Acceptor phase: V_a = 50 µL, pH 13 (except B); donor phase: V_d = 350 µL; pH 1 (except A), 0 % NaCl (w/v) (except C); T_{ext} = 25°C (except D); agitation speed 600 rpm (except E); extraction time 30 min (except F).

3.1.7. Application of the defined conditions to the extraction of AMPAs

According to results obtained for the extraction of PMPA, the extraction conditions were set as follows to study the extraction of other AMPAs: use of a PVDF membrane with 4 µl 1-octanol, pH values of the donor (V_d = 350 µl) and of the acceptor phase (V_a = 50 µl) were set at 1 and 14 respectively, extraction time of 60 minutes, agitation speed set at 1000 rpm, addition of 30% NaCl in the donor phase, temperature set at 40°C. The results are reported in (Fig. 3: pH=1, 60min). Exhaustive extraction was obtained for PMPA and CMPA but as expected the extraction recovery decreases when the analytes become more polar (extraction recoveries of only 17% for EMPA). Therefore, two parameters were reevaluated: the use of more acidic conditions (pH = 0) in the donor phase to limit as much as possible the ionization of the AMPAs and thus to favor their transfer in the SLM and a longer extraction times,

the 60-minute extraction time having been evaluated with PMPA for which equilibrium can be reached more quickly than for other compounds. As shown by results reported on Fig. 3, the use of more acidic conditions tends to decrease the extraction recoveries. In return, the increase of the extraction time favors the extraction of the most polar analytes, particularly for iPrMPA. In these conditions, extraction recoveries close to 100 % for PMPA, CMPA, and iBMPA and to 80% for iPrMPA were obtained, the extraction recovery of EMPA (the most polar analyte) being close to 25%.

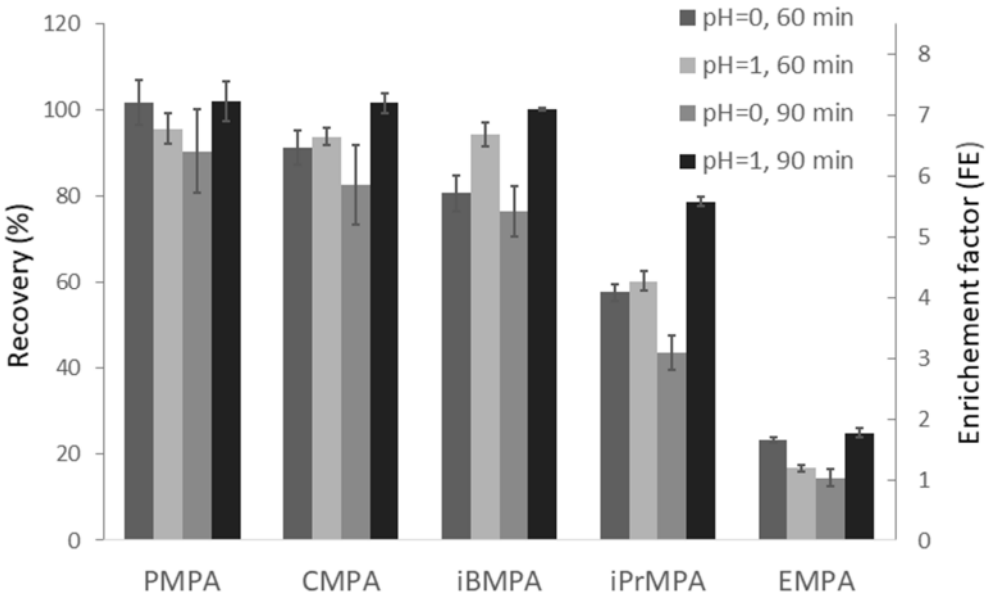

Fig. 3: Effect of the donor pH and the extraction time on the PALME recoveries and the EFs of the five studied analytes (100 ng mL^{-1}) in pure water (n=3). Extraction conditions: 4µl of 1-octanol in PVDF flat membrane (SLM); donor phase: pH = 1, V_d= 350 µL; 30% NaCl (w/v); acceptor phase: pH 14; V_a = 50 µL; 1000 rpm; T_{ext} = 40 ◦C. (Initial optimized values for donor pH and extraction time were 1 and 1H00 respectively).

Thus, while previous studies recommend the application of PALME to extract relatively non-polar compounds (log P greater than 2) [14], we showed that it was possible to obtain extraction with yields greater than 75% for compounds with log P between -0.5 and 0.8. However, all the enrichment factors do not exceed 7 as shown in Fig. 3, which is the maximum theoretical enrichment factor with this PALME set-up (V_d=350 µL, V_a= 50 µL). All the previous works with PALME were performed with a system of plates giving a maximum enrichment factor of 5 (V_d=250 µL, V_a= 50 µL) except in one study when the V_d was set at 400 µL thus giving rise the possibility to reach an EF of 8 [22]. While this has been deemed sufficient for most pharmaceutical and biomedical application [32], the limitation of PALME regarding the low resulting enrichments factors was recently pointed out by authors working on the label-free detection of ochratoxin in wine [25]. For the trace level determination of AMPAs in environmental samples high enrichment factors are requested [9]. So, in order to increase them, the volume of the donor phase was increased by modifying the original assembly. The initial donor 96-well plate with a well volume of 0.5 mL was replaced by one with a volume of 2.2 mL. The extraction of all the targeted compounds was then performed using this new donor plate (V_d was set at 2.12 mL) and by keeping other extraction conditions except the extraction duration. Indeed, the extraction was

carried out during 90 minutes as before but also during 120 minutes to compensate the larger size of the device that results in longer diffusion distance for the target compounds. As shown by results reported on Fig. 4, this larger extraction duration was not necessary for the less polar compound, PMPA that reaches equilibrium in less than 90 minutes in both cases. In return, this increase in extraction time favors the extraction recoveries for the three more polar AMPAs (iBMPA, iPrMPA and EMPA). Moreover, these conditions lead to enrichment factors greater than 30 for the three least polar compounds, greater than 10 for iPrMPA and of the order of 5 for EMPA.

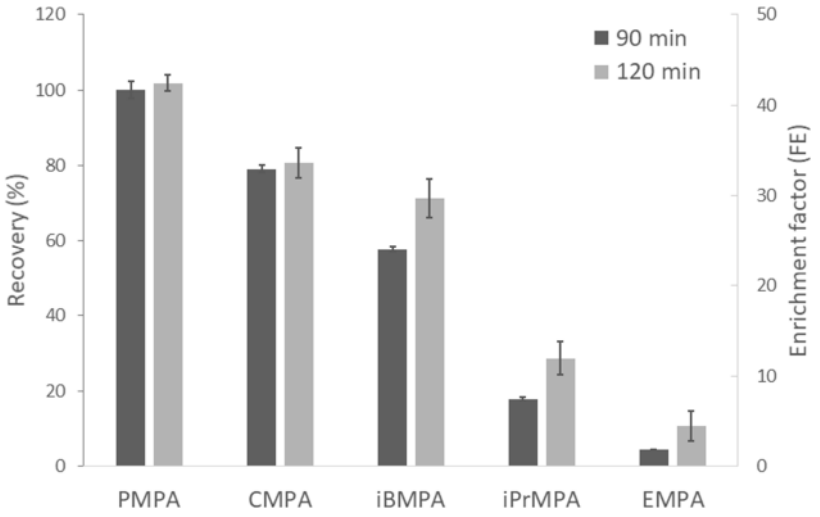

Fig. 4: Effect of the extraction time on the PALME recoveries and on the EFs for the five studied AMPAs (100 ng mL^{-1}) in spiked pure water (n=3). Extraction conditions: 4 µl of 1-octanol in PVDF flat membrane (SLM); donor phase: pH = 1, V_d = 2120 µL; 30% NaCl (w/v); acceptor phase: pH = 14; V_a = 50 µL; 1000 rpm; T_{ext} = 40 ◦C.

The comparison between the best results obtained for of PMPA using PALME to those obtained using HF-LPME [8, 9] is provided in Table. S4. Almost all the fixed parameters were the same in the two techniques such as the nature of solvent, the pH values of the donor and acceptor fixed with HCl (pH=1) and NaOH (pH=14) respectively, temperature, amount of NaCl added in the donor phase and the need of a relatively strong agitation. Altogether, its proof the possibility of a simple transfer and quick development of PALME methods starting from the abundant conditions already described for HF-LPME for numerous applications. An improvement brought by the method developed in this study was the replacement of the PP membrane by the PVDF membrane which permits a quicker impregnation with the selected solvent and a better transfer of the PMPA. Moreover, the high extraction recoveries obtained for three among the five studied compounds provided high-enrichment factors despite to the relatively low ratio between V_d and V_a, as compared to HF-LPME [44]. An enrichment factor close to 43 was obtained for PMPA with a sample volume of 2.12 mL which is comparable to its enrichment factor with HF-LPME (85) obtained with a sample volume of 6 mL (≈ 3 times the sample volume used in PALME) [8]. Finally, it is important to underline the fact that, although the method developed in PALME is longer (120 minutes) than the HF-LPME (50-60 minutes), the possibility to process 96 samples simultaneously considerably reduces the processing time before analysis when a large number of samples are to be analyzed.

3.2 Figures of merit of the PALME-LC-MS/MS method

To evaluate the performances of the developed PALME method, the extraction recoveries and the linearity of the method were assessed at 5 concentration levels in the range of 0.5 to 100 ng mL^{-1} for all the targeted AMPAs (except for EMPA, 5–100 ng mL^{-1}) in pure water. The results are given in Table. 1. Excellent linearity was obtained with a R^2 value in the range of 0.9973 to 0.9998. Repeatability was measured for all target analytes at all the different levels of concentrations. Acceptable RSD values on recoveries (n=15, except for EMPA n=12) in the range of 8.28–10.90 % were obtained thus indicating the good repeatability of the whole method.

3.3 Application to environmental samples

For the first time, PALME was applied on three types of environmental samples consisting in a simulated waste water, a river water, and aqueous soil extracts. Fig. 5 presents the EFs obtained by applying the optimal extraction conditions previously optimized (extraction during 120 minutes) except for V_d that was reduced to 1950 µl to ensure a better stability and avoid cross contamination when agitating. For all compounds, the extraction recoveries were rather the same for the different types of water samples (pure, river and simulated waste water), thus allowing to maintain satisfactory EFs for these three types of water.

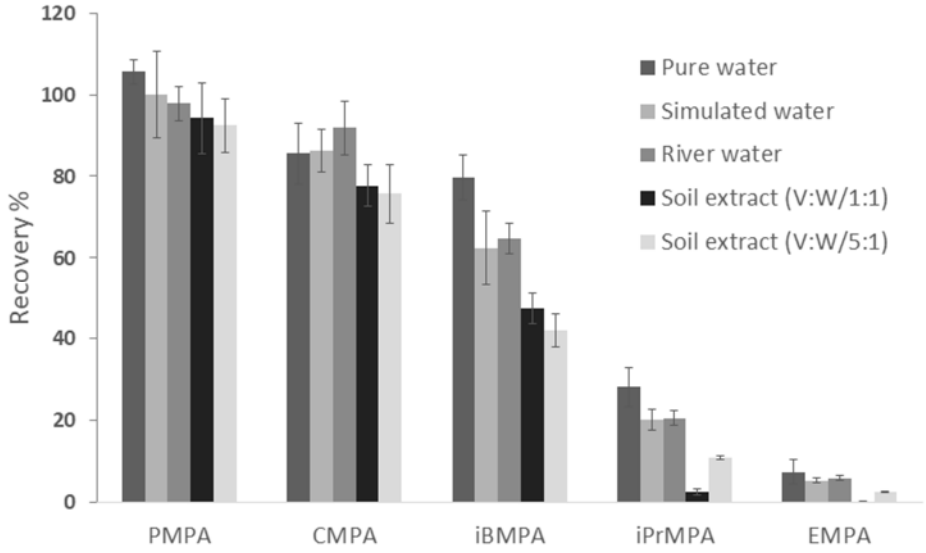

Fig. 5: Extraction recoveries obtained with PALME of the five targeted AMPAs (0.1 µg mL^{-1}) in different aqueous matrices (n=3). Constant parameters: 4 µl of 1-octanol in PVDF flat membrane (SLM); donor phase: pH 1, V_d = 1950 µL; 30% NaCl (w/v); acceptor phase: pH 14; V_a = 50 µL; 1000 rpm; T_{ext} = 40 ◦C.

In addition to the application of the PALME method to water samples, it appears interesting to apply it to aqueous soil extracts as soil samples proved to be of critical importance in confirming the use of chemical warfare agents in real conflicts [43, 44]. The efficiency of the methods applied to the extraction of AMPAs is soil-type dependent as already reported [17, 36, 45, 46]. As the development of an extraction method from soil was not the objective of this study, a soil sample was subjected to two different extraction methods previously reported for the extraction of some AMPAs [36, 37] (and detailed in section 2.2) to obtain an aqueous soil extract that was further spiked with AMPAs to evaluate the potential of PALME on this type of sample. These two methods differ mainly in the ratio of the volume of water to the amount of soil treated.

The extraction recoveries with PALME of the less polar AMPAs from extract aqueous soil samples were mostly the same as from pure water. However, an important decrease of the recoveries of the very polar compounds was observed. Only 0.21% and 2.56% were obtained for the extraction of EMPA and iPrMPA respectively from the first type of aqueous soil extract. These recoveries are improved (to 2.70% and 10.97% for EMPA and iPrMPA respectively) when applying PALME on the second type of aqueous extract soil. This result was predictable since the matrix effect was reduced when using a ratio of 5 mL of water to 1g of soil compared to the 1:1 (V/W) ratio. Soils are known to be very rich in humic acids and the dilution of the samples reduces the matrix effect they cause. Many extraction methods of soils reported in the literature are performed with a ratio of volume of water per mass of soil superior to 1. Some studies reported the determination of AMPAs in aqueous extracts of soil prepared with higher ratios (up to 7) [49]. However, despite the fact that high extraction recoveries can be expected with these conditions, the sensitivity of the final methods is affected because of this high dilution rate. Hence, a compromise between recoveries and sensitivity must be found.

The application of PALME to the most diluted soil extract leads to higher extraction yields than for the less diluted one but it is obvious that the EFs obtained on the less diluted extract are higher and make the method more efficient despite the lower yields except for EMPA. The obtained EFs with the first type of aqueous extract soil are 36.76, 30.31, 18.52, 1.04 and 0.08 for PMPA, CMPA, iBMPA, iPrMPA and EMPA respectively. The application of PALME to the second type of aqueous extract soil is only interesting for EMPA since an EF of 1.06 is obtained in these conditions.

The LOQs estimated for the studied environmental samples are reported in Table 2 except for EMPA for the first aqueous soil extract type which was not applicable for study since EF is lower than 1. These LOQs obtained for the five AMPAs are very low in particular for the less polar compounds. They are similar for river water, simulated waste water as for the pure water. Higher LOQs were obtained for the aqueous soil extracts which is normal given the complexity of these samples.

The LOQs obtained for almost all the analytes in water (0.009-1.210 ng mL^{-1}) and soil (0.016-0.515 ng g^{-1}) were lower than those previously obtained with different methods listed in Table 3, several of which were applied in a pure water [8,12,50,52]. These reported methods were performed with different types of analytical instrument and based on different extraction concepts such as the two phase HF-LPME (including a derivatization step) [48, 49], the three-phase HF-LPME [8, 9] or off-line SPE [46, 47] and on-line SPE [6, 17]. In addition to the high sensitivity of the current method compared to the other reported methods that were not all applied to real samples, PALME has also demonstrated to be a simple and quick method with a relatively low consumption of samples, since it was performed in one step with a short sample throughput time (37.5 sec) on samples of water and soil not exceeding 2mL and 2 g, respectively.

If the power of PALME to filter the phospholipids from human plasma was already demonstrated [28], we succeed to apply this technique on aqueous soil extracts without any pretreatment such as the use of expensive syringe filters. Moreover, the centrifugation step could be abandoned in favor of simple decantation. The chromatograms, obtained after the PALME-LC–MS/MS for a spiked aqueous soil extract (V:V/1:1), are presented in Fig. 6 (TIC and MRM signals of each analyte). The resultant acceptor phase obtained after PALME was sufficiently cleaned for a direct injection in LC-MS, thus allowing the quantification of the studied AMPAs at low concentrations.

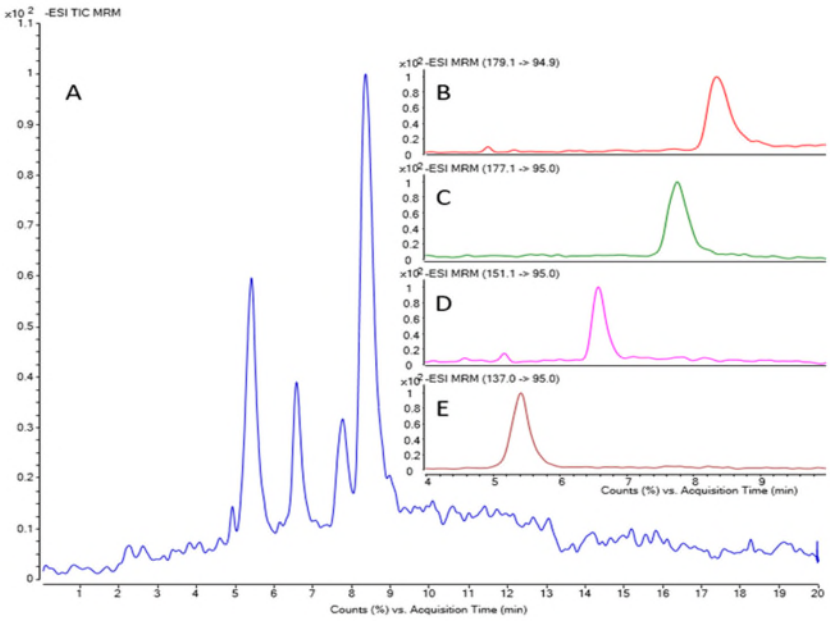

Fig. 6: Chromatogram of the target analytes obtained after from the application of PALME to aqueous soil extracts 1:1 (V/W) spiked at low concentrations ([PMPA] = 0.05 ng mL^{-1}, [CMPA] = 0.05 ng mL^{-1}, [iBMPA] = 0.1 ng mL^{-1} and [iPrMPA] = 1 ng mL^{-1}) and analysis by LC–MS/MS in negative ionization mode: (A) TIC of MRM transitions and MRM mode for (B) PMPA (m/z 179.1→ 94.9), (C) CMPA (m/z 177.1 → 95.0), (D) iBMPA (m/z 151.1 → 95.0), (E) iPrMPA (m/z 137.0 → 95.0). Extraction conditions: see Fig 5

4. Conclusion

This study highlights the potential of PALME as a preconcentration technique for the analysis of polar acidic compounds such as AMPAs. We demonstrated that it could be a serious alternative of HF-LPME by overcoming the major drawbacks of the latter. The workflow is simple and the availability of the 96-well plate technology facilitates its automation and thus its implementation in routine laboratories. Furthermore, this work has shown the possibility of easy transfer the extraction conditions of a HF-LPME method to PALME which could open the door to an easy development of many applications with PALME from the extensive HF-LMPE data available in the literature.

Three major limitations described by the PALME inventors have been overcome:(i) EFs that can be higher than 5 (ii) including for polar compounds [32] while using commercially available plates without the need to change the membrane material or use a complex mixture of organic solvents to optimize the chemical composition of the SLM [23].

We also showed the potential of PALME as a cheap green analytical high-throughput sample preparation technique for environmental applications, since only 768 µL (<1mL) of organic solvent was used to treat 192 samples in 120 minutes (equivalent to 37.5 sec/sample) with a cost that does not exceed 0.2 € per sample. In addition, the excellent sample clean-up of the technique was revealed since the acceptor phases obtained after the PALME of the aqueous soil extracts were clean and this without passing them in filter syringes as it is required for many other techniques. Thus, reducing also the workflow for this type of samples. This technique should therefore enable response laboratories to better manage the large number of samples expected in the event of a large-scale nerve agent exposure.

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Table. 1: Linearity and repeatability of the PALME-LC–MS/MS method for AMPAs in pure water.

Analyte	PMPA	CMPA	iBMPA	iPrMPA	EMPA
Concentration range (ng mL^{-1})	0.5-100	0.5-100	0.5-100	0.5-100	5.0-100
Recovery (%)	100.83	80.35	71.25	28.28	10. 61
Enrichment factor	42.75	34.07	30.21	11.99	4.50
RSD (%)*	10.90	10.06	9.80	9.40	8.28
Equation	y = 42.746x + 37.9	y = 34.069x + 42.089	y = 30.212x + 19.065	y = 11.9870x - 3.5538	y = 4.4969x - 3.8876
linearity (R^2)	0.9998	0.9993	0.9998	0.9973	0.9991
Limit of quantification (ng mL^{-1})	0.009	0.012	0.020	0.029	1.141

*: The overall relative standard deviation values of all the concentrations level for each compound (n= 15, except for EMPA n=12) were calculated after the verification of the homogeneity of the RSD values with the Cochran's test (g0.95).

Table. 2: Limits of quantification (S/N = 10) obtained for each AMPA in pure water, simulated waste water, river water and aqueous soil extracts after PALME and LC–MS/MS analysis. n.a.: not applicable.

Analyte	Pure water (ng mL^{-1})	Simulated Waste Water (ng mL^{-1})	River water (ng mL^{-1})	Soil 1:1 (V/W) ratio (ng g^{-1})*	Soil 5:1 (V/W) ratio (ng g^{-1})**
PMPA	0.009	0.011	0.013	0.016	0.075
CMPA	0.012	0.012	0.009	0.017	0.090
iBMPA	0.020	0.023	0.022	0.025	0.135
iPrMPA	0.029	0.041	0.035	0.515	0.625
EMPA	1.141	1.210	1.196	n.a	6.810

*1 ng/g is equivalent to 1 ng mL^{-1} assuming an exhaustive aqueous extraction of AMPAs from soil samples.

**1 ng/g is equivalent to 0.25 ng mL^{-1} assuming an exhaustive aqueous extraction of AMPAs from soil samples.

Table. 3: Comparison of the current PALME method with other sample preparation techniques for the determination of AMPAs in water and soil samples

Sample matrix	Analytes	Technique	Sample volume/amount	Extraction time	Analytical method	LOD*, LOQ** (ng mL^{-1})	Ref
Pure Water	EMPA, iPrMPA, iBMPA, CMPA, PMPA	Direct injection	5–20 µl	0 min	LC-APCI-MS (SIM)	10–100*	[50]
Deionized water	EMPA, iPrMPA , PMPA	HFLPME (two-phase, derivatization)	3 mL	45 min	GC/MS (scan)	0.16, 0.03 and 0.05*	[51]
Triple distilled water	IPrMPA, PMPA	HFLPME (two-phase, derivatization)	1.5 mL	150 min	GC/MS (SIM)	100 and 500*	[52]
Tap water	EMPA, iPrMPA, iBMPA, CPMA, PMPA	HFLPME (two-phase, derivatization)	4 mL	70 min	GC/MS (NCI,SIM)	0.1-10**	[53]
Pure water	iPrMPA, iBMPA, PMPA	HFLPME (three-phase)	6 mL	60 min	LC-ESI-MS^n (EIC)	2.0,2.0 and 0.1*	[8]
River water + Simulated Waste Water	EMPA, iPrMPA, iBMPA, PMPA	HFLPME (three-phase)	3 mL	50 min	LC-ESI-MS (SIM)	0.013-6.3**	[9]
Tap water + natural water + simulated waste water	EMPA, iBMPA, PMPA	Off-line SPE (Ba/Ag/H) + On-line SPE (PGC)	10 mL	> 15 min	LC-ESI-TOF-MS	3.4-7.2**	[6]
Pure water + river water + simulated waste water	EMPA, iPrMPA, iBMPA, CMPA, PMPA	PALME	1.95 mL	37.5 sec (120 min for 96*2 samples)	LC-ESI-MS/MS (MRM)	0.009-1.210**	Actual study

Different soil types	iPrMPA, PMPA	Direct injection	1 g	0 min	LC-ESI-TOF-MS	/	[33]
Aqueous extracts from different types of soil	EMPA, iPrMPA, PMPA	SPE with 3 On-Guard cartridges (Ba/Ag/H)	1.5 g	n.g	CE/UV	166-333 ng g^{-1}* (a)	[49]
Different soil types	EMPA, iPrMPA, PMPA	SPE (SCX) + derivatization	2g	> 1 hour	GC/MS in scan mode	120-180 ng g^{-1}*	[48]
Soil	EMPA, iBMPA, PMPA	SPE (MIP)	10 g	> 1 hour	IC/conductimetry	/	[54]
Aqueous extracts from different types of soil	EMPA, iPrMPA, iBMPA, CMPA, PMPA	Off-line SCX SPE (Ba/Ag/H) + On-line SPE (zirconium)	1 g	> 15 min	LC-ESI-MS (scan)	0.15–1.5 ng g^{-1}**(a)	[16]
Aqueous extracts from park soil	iPrMPA, iBMPA, CMPA, PMPA	PALME	1.95 g	37.5 sec (120 min for 96*2 samples)	LC-ESI-MS/MS (MRM)	0.016-0.515 ng g^{-1} **(a)	Actual study

n.g: not given

a: Calculated assuming 100% soil extraction recovery of AMPA's into the aqueous phase

35F: 1-(diazomethyl)-3,5-bis(trifluoromethyl)benzene; NCI: negative Chemical ionization; SIM: single ion monitoring; EIC: extracted ion mode; MRM: multiple reaction monitoring, PGC: porous graphitic carbon; IC: ion chromatography; SCX: strong cation exchange

Electronic Supplementary Information

Parallel artificial liquid membrane extraction of organophosphorus nerve agent degradation products from environmental samples

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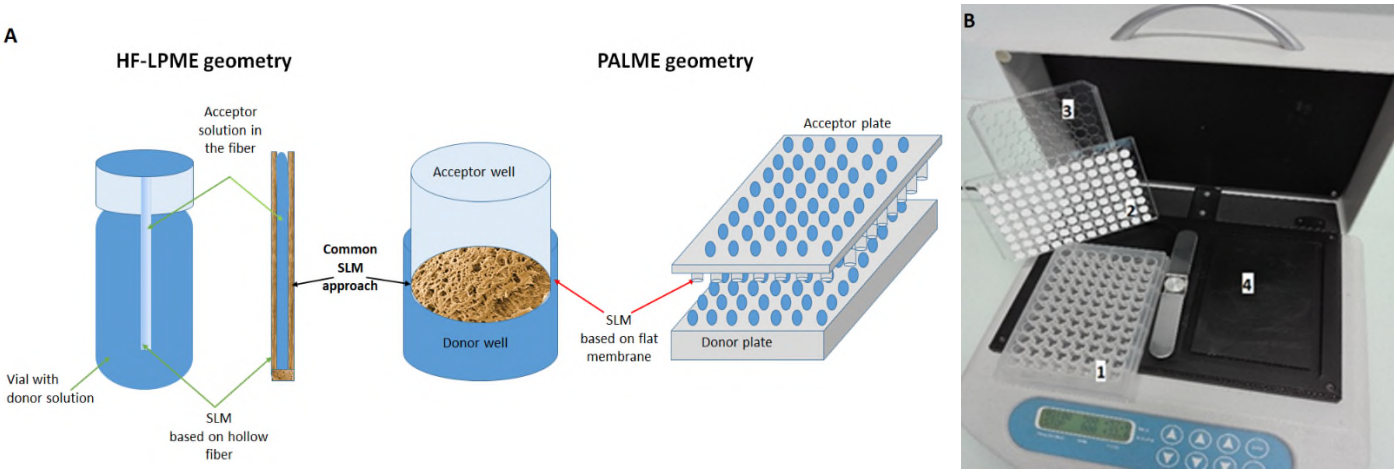


Fig. S1. Schematic illustration of the difference of geometry between HF-LPME and PALME (A). Picture of the hole set-up used for PALME (B): the donor plate (1), the acceptor plate (with white filter PVDF membranes) (2), the lid (3) and the bi-plate shaker (4).

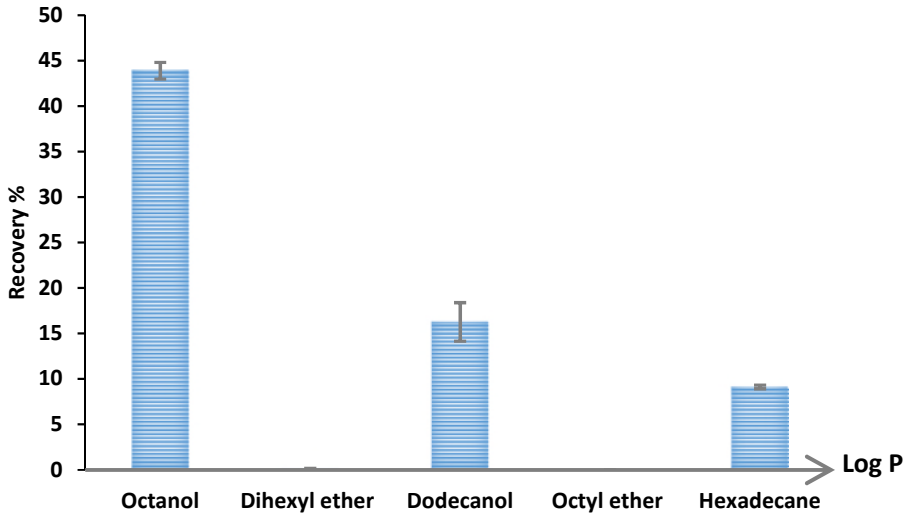
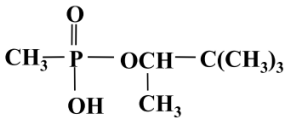
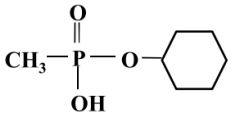
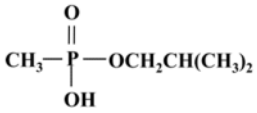
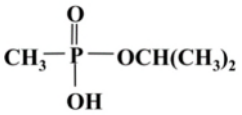
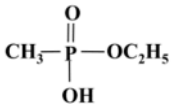


Fig. S2. Effect of the organic solvent on the extraction recovery of 0.1 µg mL^{-1} of PMPA from pure water (n = 3). Extraction conditions: membrane material: PVDF; donor phase: pH 1, Vd = 350 µL, 0 % NaCl (w/v); acceptor phase: pH 13, Va = 50 µL; immersion of 4 µL of different organic solvents; extraction time: 30 min; temperature: 25 ◦C and stirring speed: 600 rpm.

Table. S1. Physicochemical properties of the studied alkyl methylphosphonic acids.

Analytes	Chemical Structures	Molecular weight (g/mol)	Log P* /log P **	pKa***
PMPA		180.18	0.67 to 1.409 / 0.8±0.6	2.06
CMPA		178.17	0.62 to 1.037 /0.6±0.6	1.97
iBMPA		152.13	-0.03 to 0.564 /0.0±0.6	2.03
iPrMPA		138.10	-0.56 to 0.101 /-0.5±0.6	1.96
EMPA		124.08	-0.91 to -0.310 /-0.8±0.6	1.99

*Range obtained from https://chemicalize.com, https://pubchem.ncbi.nlm.nih.gov and www.chemspider.com.
**values from Røen et al., 2014 [1]
*** obtained from https://chemicalize.com.

Table. S2. Parameters applied in ESI-MS detection (MRM mode)

Analyte	Capillary voltage (V)	Precursor (m/z)	Fragmentor (V)	Quantifier ion (m/z)	CE (V)
PMPA	2500	179.1	136	94.9	18
CMPA	2500	177.1	112	95.0	18
iBMPA	3000	151.1	112	95.0	14
iPrMPA	3500	137.0	78	95.0	14
EMPA	2500	123.0	44	94.9	10

Table. S3. Characteristics of the studied membranes [2][3].

Characteristics	PVDF IMOBILON p	Accurel PP 1E (R/P)
Wall thickness	100-130 µm	100 µm
Diameter	6 mm	6 mm
Pore size	0.45 µm	0.1 µm
Membrane porosity	68 %	69%

Table. S4. Comparison between previous HF-LPME and PALME of PMPA in pure water

Microextraction technique and Reference		**Tak et al** [4] **HF-LPME**	**Desoubries et al** [5] **HF-LPME**	**Actual work PALME**
Physical characteristics	Type of membrane	Hollow fiber	Hollow fiber	Flat membrane
	Membrane material	Accurel Q 3/2 PP	Accurel Q 3/2 PP	Immobilon®-P PVDF
	Thickness (µm)	200	200	100-130
	Pore size (µm)	0.20	0.20	0.45
	Contact area of the SLM (mm2)	n.g	56.83	28.27
	Porosity	69.0 %	69.0 %	68.4 %
Optimal conditions	Preconditioning of the membrane	Yes (sonication)	Yes (sonication)	No
	Vd (mL)/ Va (µL)	6/8	3/6	2.12/50
	pH (donor phase) /pH (acceptor phase)	1 (HCl) / 14 (NaOH)	1 (HCl) /14 (NaOH)	1 (HCl) /14 (NaOH)
	NaCl (%)	10	30	30
	Solvent (immersion time)	1-Octanol (20s)	1-Octanol (5s)	1-Octanol (instantly)
	Stirring speed/Agitation (rpm)	900	600	1000
	Temperature	RT	42°C	40°C
	Extraction time (min)	60 min/sample	50 min/sample	120 min for 96*2 samples (37.5 sec/sample)
[2]Performance	FE (max achievable) = Vd/Va	750	500	42.4
	FE obtained	85 ± 8	225 ± 30	42.75 ± 4.66
	Recovery %	11.33 %	45 %	100.83 %
	LOD or LOQ of PMPA in pure water	LOD (S/N≥5): 0.1 ng/mL	LOQ (S/N≥10): 0.013 ng/mL	LOQ (S/N≥10): 0.009 ng/mL

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