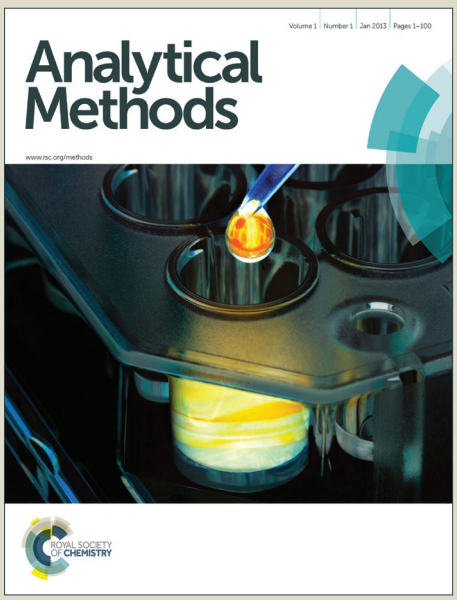
# Analytical Methods

Accepted Manuscript





ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

# A Simplified Vortex−Assisted Emulsification Microextraction Method for Determining Personal Care Products in Environmental Waters by Ultra−High−Performance Liquid Chromatography

Providencia González-Hernández, Verónica Pino, Juan H. Ayala, Ana M. Afonso*

*Departamento de Química, Área de Química Analítica, Universidad de La Laguna (ULL), La Laguna (Tenerife), 38206 Spain*

## Abstract

A vortex-assisted emulsification microextraction (VAEME) procedure has been evaluated for the determination of ten personal care products (PCPs), including seven preservatives (parabens), two UV filters (benzophenones), and one disinfectant (triclosan), in environmental waters. The method is utilized in combination with ultra-high performance liquid chromatography (UHPLC) and UV detection. The liquid-phase microextraction method results quite simple because it only needs one extractant solvent (200 µL of trichloromethane under optimum conditions) and it completely avoids the use of any dispersive solvent neither surfactants to help the emulsification. The optimized method ensures the correct emulsification by simple application of 3 min of vortex to 8 mL of aqueous sample at pH 5 containing 15% (w/v) of sodium chloride, followed by centrifugation (5 min at 3500 rpm), droplet sampling using a syringe, droplet solvent evaporation, and reconstitution with 100 µL of a mixture of acetonitrile:water (35:65, v/v) before UHPLC injection. The overall extraction time is roughly 10 min, and the chromatographic time ~12 min. The optimized method was validated, presenting average relative recoveries of 112%, average real extraction efficiencies of 82.7%, inter-day precision values with relative standard deviation (RSD, in %, for n = 9) values lower than 10%, and enrichment factors between ~20 and ~100,

for a spiked level of 3.75 µg·L$^{-1}$. Limits of detection down to 0.03 µg·L$^{-1}$ were also obtained. The method satisfactory performed with environmental water samples with different nature and complexity.

**Keywords*:*** Personal care products / Microextraction / Vortex-assisted emulsification / Ultra-high performance liquid chromatography / Environmental waters / Dispersive solvent-free

*Corresponding author: Tel. +34 922318039. Email: aafonso@ull.es

P. González-Hernández mgonzalh@ull.es
V. Pino veropino@ull.edu.es
J.H. Ayala jayala@ull.es

# 1 Introduction

Pharmaceutical and personal care products (PPCPs) constitute a wide group of organic chemical compounds used as drugs, in cosmetic products, or with agricultural and food purposes.[1] Among PPCPs, cosmetic ingredients, commonly known in the scientific community as personal care products (PCPs), are a subcategory of less studied compounds, widely employed in creams, gels, fragrances, sunscreens, etc. PCPs are in general classified in six major groups: UV filters, preservatives, disinfectants, musk fragrances, insect repellents and siloxanes.[2] Its growing and intensive use is accompanied by an overload in the removal capacity of wastewater treatment plants (WWTPs). Thus, WWTPs are an important source of incorporation of PCPs into the environment.[3,4] PCPs are also obviously present in the environment due to its direct incorporation by human aquatic leisure activities.[5]

The increasingly significant presence of PCPs in diverse environmental samples (superficial waters, wastewaters, sediments, air…) has attracted scientific interest while alerting for potential risks.[6,7] Some PCPs have recently been classified as emerging contaminants, even able to act as endocrine disruptors.[8,9] Therefore, the development of sensitive and selective methods devoted to the determination of PCPs at trace levels in environmental samples is of high interest.[2]

The determination of PCPs by gas chromatography (GC) coupled to mass spectrometry (MS) detection is limited to volatile and semivolatile compounds such as siloxanes[10,11] and musk fragrances[12,13]. Other PCPs usually require a derivatization step prior to GC analysis.[14-17] High−performance liquid chromatography (HPLC) has been used for the determination of different kinds of PCPs.[18-20] Recent applications for PCPs that utilize ultra-high performance liquid chromatographic (UHPLC), mainly focused on preservatives, UV filters and disinfectants, has also been reported.[21-24]

In any case, low levels of PCPs in environmental waters necessarily imply the utilization of extraction/preconcentration techniques prior to the chromatographic analysis. It results contradictory, from an environmental point of view, the utilization of large amounts of toxic organic solvents in these previous stages, as it commonly happens with conventional extraction techniques such as liquid−liquid extraction (LLE) and even solid−phase extraction (SPE). Recently, green approaches in sample preparation are clearly shifted to the elimination or at least minimization of such solvent consumption.[25,26]

Dispersive liquid–liquid microextraction (DLLME) was developed by Rezaee *et al.* in 2006.[27] It is based on the utilization of a mixture of a water−immiscible extractant solvent (normally an organic solvent) and a water−miscible polar dispersive solvent (normally methanol, acetonitrile or acetone). Analytes experience enrichment in the low volume of extractant solvent (in the order of microlitres) which is dispersed into the bulk aqueous solution with the aid of the dispersive solvent, and further separated by centrifugation. The advantages of DLLME are simplicity of the process, high enrichment factors and recoveries, and mainly short extraction times compared to other liquid–phase microextraction (LPME) modes.[28,29] Among the disadvantages, it can be cited that the dispersive solvent can solubilize minimum amounts of the extractant solvent in the process, consequently provoking a decrease in the overall extraction efficiency.

In last years, modifications of DLLME have been developed with the purpose of: (*i*) automation[30]; (*ii*) replacing the dispersive solvent by less toxic dispersive agents[31,32], or (*iii*) avoiding the necessity of a dispersive solvent. Following this last trend, some recent works have described how to disperse the extractant solvent in the aqueous solution without the need of a dispersive solvent[33], for example by the application of a current of

air (air assisted liquid−liquid microextraction: AA−LLME)[15], by the application of ultrasounds (ultrasound−assisted emulsification microextraction: USAEME)[16], or by the application of vortex (vortex−assisted emulsification microextraction: VAEME)[34]. The energy generated during USAEME is not uniform, and consequently the emulsification is not reproducible. Furthermore, analyte degradation may occur.[33] Giving the low applications of AA−LLME, the most successful variant is VAEME.

VAEME was first described by the group of Psillakis in 2010.[34] The main success of this method is that the generated emulsification by vortex is homogenous, while generating a high surface contact between the extractant solvent and the aqueous sample. Moreover, it totally avoids the necessity of a dispersive solvent, in this way requiring a single extractant solvent to obtain quantitative recoveries without the loss of any extractive efficiency, as it happened in conventional DLLME. Applications of VAEME include the determination of pesticides[35,36], phthalate esters[37], phenols[38] and metals[39]. Recently, the method has also been proposed for the determination of octanol/water partition coefficients.[40]

It is important to note that not all reports related with the use of vortex in LPME, sometimes even named as VAEME, are necessarily dispersive solvent−free[41], and indeed they utilize an extra aid for emulsification such as acetonitrile[42,43], methanol[44], or surfactants[45-51].

The main purpose of the present work is to utilize VAEME as novel microextraction technique, without the need of surfactants or any co−solvent rather than vortex in the microextraction procedure, for the determination of a group of ten PCPs. To the best of our knowledge, this is the first report on the utilization of VAEME in combination with UHPLC for determining several PCPs of different nature, including seven preservatives (specifically parabens), two UV filters, and one disinfectant, in

environmental waters. In the literature, the utilization of neat VAEME for PCPs has only been reported before for 6 UV filters and GC-MS[52], requiring a derivatization step (30 min, 75 ºC) after VAEME and before GC injection.

# 2 Experimental

## 2.1 Chemicals, reagents and materials

Ten PCPs were studied in this work. Methylparaben (MPb), ethylparaben (EPb), propylparaben (PPb), *iso*butylparaben (*i*BPb) and triclosan (Tr) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany); butylparaben (BPb), benzylparaben (BzPb), benzophenone (BP), and benzophenone-3 (BP-3) were supplied from Sigma-Aldrich (Steinheim, Germany); and *iso*propylparaben (*i*PPb) from Alfa Aesar (Karlsruhe, Germany). The purity was greater of 99% in all cases, except for *i*PPb, which was 98%.

Stock solutions were prepared in methanol, at concentrations between 800 and 4200 $mg \cdot L^{-1}$, and stored protected from light at 4 ºC. Working standard solutions were prepared every fifteen days by dilutions of the stock solutions with a mixture of acetonitrile/water at 65/35 (v/v), and filtered using Chromafil® Xtra PET 20/25 filters (0.20 µm) from Macherey Nagel (Düran, Germany).

Deionized water was obtained using a water purification system Milli-Q gradient A10 from Millipore (Watford, UK). Methanol of HPLC grade was from Scharlau (Barcelona, Spain) and acetonitrile of LC-MS grade from VWR International (Barcelona, Spain). The solvents: octanol, decanol, trichloromethane and tetrachloroethylene, were supplied from Sigma-Aldrich, while dichloromethane was acquired from Scharlau. Sodium chloride (purity ≥99.5%) was also acquired to Sigma-Aldrich. The surfactants cetyltrimethyl ammonium bromide (CTAB), polyoxyethylene-

10-lauryl ether ($C_{12}E_{10}$), and hexadecylpyridinium chloride monohydrate ($C_{16}$PyCl) were purchased from Sigma-Aldrich, while sodium dodecyl sulfate (SDS) was acquired to Merk (Darmstadt, Germant). The ionic liquid-based surfactant 1-hexadecyl-3-methylimidazolium bromide ($C_{16}$MImBr) was synthesized and fully characterized according to a previous work.[53]

A vortex Reax Top from Heidolph (Schwabach, Germany) and a centrifuge model 5720 from Eppendorf (Hamburg, Germany) were also utilized in the microextraction procedure. The solvent-exchange step was carried out using an air-current assisted by vacuum, with the Visiprep™ system of Supelco (Bellefonte, PA, USA). Mobile phases were always filtered using Durapore filters of Millipore of 0.22 µm to avoid problems in the UHPLC system.

## 2.2 Sample collection

All water samples were collected in Tenerife (Canary Islands, Spain). The swimming pool waters were sampled in two public pools. The seawaters were sampled in two different beaches, located at the north and south of the island, respectively. Tap water taken at the laboratory was also analyzed. Two more samples were taken from a WWTP, and collected in different days. Wastewaters were directly sampled in the plant. In all cases, sampling was carried out avoiding the formation of bubbles, and using clean amber glass bottles of 100 mL in volume. They were also kept in a portable fridge until they reached the laboratory, and then kept in the dark at 4 ºC for no more than 48 h. before being analyzed. Before analysis, the ionic strength was adjusted by addition of sodium chloride.

## 2.3 Instruments

Chromatographic analysis was carried out using a UHPLC 1260 Infinity Series from Agilent Technologies with a quaternary pump, and a Rheodyne 7725i injection valve with a loop of 5 μL. The chromatographic column was a ZORBAX Eclipse Plus C18 (2.1 mm×50 mm×1.8 μm) purchased from Agilent Technologies. The detector was a Vis-UV ProStar 325 LC Detector Series supplied from Varian (Palo Alto, CA, USA).

The optimum separation required a binary mobile phase composed of acetonitrile and water with a 0.1% (v/v) of acetic acid in the aqueous phase, a constant flow rate of 0.5 $mL \cdot min^{-1}$, and a constant temperature of 25 °C. Thus, 35% (v/v) of acetonitrile was kept isocratic during the initial 5.5 minutes, followed by a linearly elution gradient from 35 to 70% (v/v) of acetonitrile in 5.5 minutes, and then kept again under isocratic conditions for 4 additional minutes. The wavelength of the detector was fixed at 254 nm from 0 to 7 minutes, and then at 289 nm during the rest of the chromatogram.

### 2.4 Procedures

All variables exerting an influence on the VAEME performance were optimized. The optimum conditions were: 8 mL of a water containing 15% (w/v) of NaCl were placed in a centrifuge tube of 30 mL in volume. Then, 200 μL of the extractant solvent were added, followed by application of 3 minutes of vortex. Finally, the tube was subjected to centrifugation during 5 minutes at 3500 rpm. The obtained microdroplet (containing extracted and preconcentrated PCPs) was introduced in a vial of 2 mL of capacity with the aid of a microsyringe, and the solvent was evaporated to dryness using a current of air assisted by vacuum. Finally, PCPs were reconstituted with 100 μL of an already filtered mixture of acetonitrile/water at 35/65 (v/v), followed by direct injection in the UHPLC.

### 2.5 Assessment of the method performance

The relative recovery (RR) was calculated as:

$$RR(\%) = 100 \cdot \frac{C_{found}}{C_{initial}} \quad \text{(Equation 1)}$$

being $C_{found}$ the calculated concentration of the PCPs using the calibration of the overall method (VAEME-UHPLC-UV), and $C_{initial}$ the spiked concentration of PCPs in water sample. In general, for microextraction methods it is expected to obtain relative recoveries around 100% if the precision of the method is acceptable.

The enrichment factor ($E_F$) of the overall VAEME-UHPLC-UV method is given by:

$$E_F = \frac{C_{droplet}}{C_{initial}} \quad \text{(Equation 2)}$$

being $C_{droplet}$ the concentration of PCPs obtained in the final droplet that is injected in the UHPLC, and so it can be calculated with the UHPLC-UV chromatographic calibration. This enrichment factor includes the preconcentration factor of the evaporation/reconstitution stage. The enrichment factor can also be calculated as the ratio of calibrations slopes, being defined as:

$$E_F' = \frac{\text{Slope calibration VAEME}-\text{UHPLC}-\text{UV method}}{\text{Slope calibration UHPLC}-\text{UV method}} \quad \text{(Equation 3)}$$

The extraction efficiency ($E_R$) of the overall method can be calculated by:

$$E_R = 100 \cdot \frac{E_F}{E_{Fmax}} \quad \text{(Equation 4)}$$

being $E_{Fmax}$ the maximum preconcentration that would be achieved if all PCPs (initially present in the water sample) were successfully transferred to the final droplet that is injected in the UHPLC. This value can be estimated from the ratio $V_{initial}/V_{droplet}$, being $V_{initial}$ the initial aqueous sample volume (8 mL).

# 3 Results and discussion

### 3.1 Chromatographic method

Ultra-high performance liquid chromatographic with UV-Vis detection was selected for the determination of the ten PCPs selected in this work. The optimum conditions for the separation were included in Section 2.3. The values of the relative standard deviation (RSD, in %) for the retention times were lower than 0.6% (n = 22).

The chromatographic calibrations were undertaken by plotting the peak-area *versus* concentration, using a range of 0.01 to 2.00 $mg \cdot L^{-1}$ for all PCPs studied. Calibrations exhibited excellent linearity with determination coefficient ($R^2$) greater than 0.997, as it can be observed in Table S1 of the Supplementary material. The detection limits (LOD) and quantification limits (LOQ) were calculated as the concentrations that provided a signal to noise ratio of 3 and 10, respectively; and verified by preparation of standards at such levels. Values of LODs ranged from 0.005 $mg \cdot L^{-1}$ for PPb and 0.056 $mg \cdot L^{-1}$ for BP-3, while LOQs ranged between 0.020 and 0.140 $mg \cdot L^{-1}$ for the same analytes.

The precision of the chromatographic method was evaluated in terms of RSD (in %) at three levels of concentration (0.1, 1.0 and 1.7 $mg \cdot L^{-1}$). At the lowest level, BP, BP-3 and Tr were not included because the studied concentration was below their respective LOQs. RSD values at the lowest level (0.1 $mg \cdot L^{-1}$) ranged from 0.43% for EPb to 1.82% for BPb, and at the highest level (1.7 $mg \cdot L^{-1}$) they ranged from 0.38% for EPb and *i*PPb, to 2.13% for Tr, showing the high repeatability of the chromatographic method. All precision values have also been included in Table S1 of the Supplementary material.

### 3.2 Screening of extractant solvents

VAEME has been selected as microextraction procedure in this work due to its simplicity, and also because it avoids the need of a dispersive solvent, overall generating a more efficient method with higher enrichment factors. A valid extractant

solvent in VAEME should meet several ideal requirements: it must generate quantitative extraction of the studied analytes, its volume should be as lower as possible, and it should be compatible with the further analytical instrument where determination is going to be accomplished.

Initially, octanol, decanol, dichloromethane, trichloromethane and tetrachloroethylene were tested as possible extractant solvents. Their most relevant physicochemical properties are shown in Table 1. To ensure compatibility of these solvents with the further determination by UHPLC, a reconstitution step was required prior injection. Thus, a volume of 200 µL of these extractant solvents containing a known amount of PCPs (250 $\mu g \cdot L^{-1}$), was subjected to evaporation until dryness followed by reconstitution with 100 µL of UHPLC mobile phase (ACN/$H_2O$ at 35/65 (v/v)), and UHPLC-UV determination. An initial volume of 200 µL was selected for these experiments, as an estimation of the maximum final volume acceptable for a microdroplet in VAEME, with respect to 8 mL of water.

Octanol and decanol were quickly discarded because the stage of evaporation/reconstitution required more than 60 minutes. The times required for evaporation of dichloromethane and trichloromethane were about ~2 minutes, while for tetrachloroethylene was ~7 minutes. The resulting recoveries of this stage (evaporation/reconstitution) can be observed in Figure 1. Clearly, dichloromethane is not a valid solvent, probably due to its high volatility, and its use is accompanied by important losses of analytes in this stage. Tetrachloroethylene was selected as possible solvent for VAEME due to its high recoveries in this stage of evaporation/reconstitution, altogether with trichloromethane. Trichloromethane was selected due to acceptable performance in this step, and also because this solvent

presents similar $logK_{OW}$ values (Table 1) to the most polar analyte, MPb ($logK_{OW}$ = 1.88). Further optimization of the VAEME method has been carried out both solvents.

### 3.3 Optimization of VAEME-UHPLC-UV

Main variables exerting an influence in the VAEME efficiency have been studied, such as: volume of extractant solvent, ionic strength of the aqueous sample, and pH of the aqueous sample. To simplify the optimization of the extraction method, the centrifugation time and velocity were fixed at 5 minutes and 3500 rpm, respectively. Higher centrifugation times and velocities are hardly needed for correct separation of the final microdroplet.

Previous experiments allowed us to fix the vortex time at 3 minutes, because longer times did not improve the extraction efficiency, and also because they are not recommended for laboratory operators.

Given the low number of factors needed in the optimization of the VAEME method, a factor by factor optimization was selected. This is also one advantage of the VAEME method: its simplicity.

In all experiments, the sample volume was fixed to 8 mL. Optimization was conducted with ultrapure water, containing the ten PCPs studied at a concentration of 12.5 $\mu g \cdot L^{-1}$.

**3.3.1 Influence of the extractant volume.** The volume of extractant solvent (tetrachloroethylene or trichloromethane) was studied from 50 to 200 µL, in order to obtain a low volume of final microdroplet while ensuring reproducibility as well as easy manipulation. Figure 2 shows the average recoveries obtained for each PCP and extractant solvent. There was not adjustment of pH, and the ionic strength was fixed with NaCl at 20% (w/v) in these initial experiments.

For both solvents, the best volume to work with was 200 µL except for BP, which was 150 µL. Higher volumes were not tried to ensure a microextraction context, and also to avoid further decreases in the enrichment factor. For tetrachloroethylene, recoveries ranged from 3.69 ± 0.31% for MPb to 114 ± 2% for BP-3, and for trichloromethane between 38.3 ± 1.7% for MPb and 108 ± 2% for BzPb.

**3.3.2 Influence of the ionic strength.** In LPME procedures, it is well-known that the addition of salts normally facilitates the handling of the final microdroplet, and also helps in increasing the extraction efficiency in many cases. Thus, the ionic strength of the initial aqueous sample was adjusted by addition of different NaCl amounts, between 0 and 20% (w/v), while keeping other VAEME variables constant: 200 µL for the extractant solvent volume and no adjustment of the pH.

Figure 3 shows the average recoveries obtained at different NaCl contents for three PCPs, selected as representative of each family of the PCPs studied. In general, best recoveries were obtained using a NaCl content of 15% (w/v), ranging from 2.90% for MPb (result not included in Figure 3) to 91.2% for Tr when using tetrachloroethylene as extractant solvent, and from 37.8% for MPb (result not included in Figure 3) to 112% for BzPb when employing trichloromethane. In any case, the effect of the NaCl content was not highly significant, particularly if compared with the pH.

**3.3.3 Influence of the pH.** The influence of the pH of the aqueous sample is evidently going to affect analytes with basic or acidic groups. It is important to select an appropriate pH, which ensures that PCPs are in their neutral forms prior to extraction. Thus, it is favored their affinity for the organic extractant solvent. The pH was studied at three values: 3, 5 and 7, attending to the nature of the PCPs selected. Other values fixed in the VAEME method were the already optimum values: 200 µL of extractant solvent and 15% (w/v) of NaCl.

Figure S1 of the Supplementary material shows the average recoveries obtained for each PCP studied, using tetrachloroethylene as extractant solvent in the example. Clearly, the best results were obtained using a pH value of 5, which was selected for further works.

### 3.4 Quality analytical parameters of the VAEME-UHPLC-UV method

From the optimization study, it is remarkable that best recoveries were obtained using trichloromethane as extractant solvent, particularly for polar analytes. In any case, several quality analytical parameters of the VAEME-UHPLC-UV method were also obtained for tetrachloroethylene, and have been included in Table S2 for comparison purposes.

For the optimum solvent, trichloromethane, calibrations were obtained by preparing aqueous standards with a concentration range between 0.63 and 25 $\mu g \cdot L^{-1}$ (depending on the PCP studied), using 8 calibration levels, and subjecting them to the overall VAEME-UHPLC-UV method (see Table 2). The obtained determination coefficients for the overall method were higher than 0.993. LODs and LOQs were calculated as the initial concentration in water that provided a final chromatographic signal to noise ratio of 3 and 10, respectively. LODs oscillated from 0.03 $\mu g \cdot L^{-1}$ for MPb to 1.65 $\mu g \cdot L^{-1}$ for Tr, while LOQs from 0.60 $\mu g \cdot L^{-1}$ for *i*BPb and 3.49 $\mu g \cdot L^{-1}$ for Tr. These values are quite low, particularly if we take into account that UV detection was used in combination with UHPLC. In the literature, the majority of recent reports utilize UHPLC in combination with MS/MS. Thus, LODs for parabens and UV filters (benzophenones) ranging from 0.4 to 4 $ng \cdot L^{-1}$ have been reported when using SPE and UHPLC-MS/MS and environmental waters[54], and from 2.5 to 5 $ng \cdot L^{-1}$ for BP-3 and Tr

in environmental waters when using stir-bar sorptive extraction (SBSE) and UHPLC-MS/MS[23].

The precision of the whole method was evaluated in terms of intra-day and inter-day repeatability (RSD in %). This study was carried out at two spiked levels: a low level (3.75 $\mu g \cdot L^{-1}$) and an intermediate level (16.2 $\mu g \cdot L^{-1}$), with respect to the concentration levels used in the calibrations. Intra-day precision was performed by 3 consecutive determinations at both levels. Their values have been included in Table 3, and they ranged between 1.0% for *i*PPb and 10% for BP at the low spiked level; and between 4.5% for BzPb and 18% for MPb for the intermediate spiked level with the exception of BP which gave a high RSD value of 25%. Inter-day precision was obtained through 3 determinations in 3 non-consecutive days, at the abovementioned spiked levels. An analysis of variance (ANOVA) was performed to determine whether there were significant differences in the results obtained by different days. The ANOVA study indicated that there were not such differences among the results obtained ($\alpha$ = 0.05). The RSD values corresponding to the inter-day precision ranged from 4.8% for BP-3 to 10% for BP at the low spiked level; and from 4.4% for *i*BPb to 7.0% for MPb for the intermediate spiked level, being again the exception BP at this level, with a high RSD value of 27% (Table 3). We observed low reproducibility performance for BP when working at relatively high spiked levels.

The VAEME-UHPLC-UV method was also evaluated in terms of extraction efficiency performance, also at the abovementioned spiked levels. It is important to distinguish between the relative recovery (RR, in %), the enrichment factor ($E_F$ or $E_F$'), and the real extraction efficiency ($E_R$, in %), as described in Section 2.5. The obtained values are listed in Table 3.

The average RR value obtained was 112% at the low spiked level, and 99.2% for the intermediate spiked level, being totally adequate for a microextraction method. The enrichment factors oscillated between ~20 and ~100 depending on the PCP, and independently on the spiked level. It can be observed the agreement in the enrichment factor values ($E_F$ and $E_F$'), independently on their calculation methods. Clearly, the experimental enrichment factor values obtained are quite close to the maximum enrichment factor, which is 80. Regarding extraction efficiency, the VAEME-UHPLC-UV method was practically quantitative for most PCPs studied, which is not necessary valid for a microextraction methods. Average $E_R$ values were of 82.7% for the low spiked level, and of 76.3% for the intermediate spiked level, for all PCPs studied. It can be also observed that low efficiencies at both spiked levels were obtained for MPb (values of 37.9 and 28.5%, respectively) and for BP (values of 35.1 and 24.7%, respectively). For MPb, reasons can be linked to its low $K_{OW}$ value (and so low affinity for an organic solvent), and for BP to its distinct nature compared to the remaining PCPs (absence of any hydroxyl group in its structure).

### 3.5 Assessment of the necessity of surfactants and/or dispersive solvents in VAEME

The main interest of the VAEME method relies on its simplicity: the method does not require a dispersive solvent and/or a co-solvent such as surfactant. However, many works in literature utilize VAEME in combination with dispersive solvents[42-44] or surfactants[45-51], as an aid in the emulsification procedure. We decided to test if these solvents were really needed in our VAEME application, perhaps to help in the improvement of the recoveries for MPb and BP.

At first, we studied if the presence of acetonitrile (a common dispersive solvent) was going to exert an influence in the VAEME performance. Studies were carried out at optimum conditions of neat VAEME with trichloromethane, but also using 500 µL of acetonitrile as dispersive solvent. The spiked concentration of PCPs in water was 12.5 $\mu g \cdot L^{-1}$. The obtained results implied slight improvements in recoveries for MPb and EPb, but mainly important decreases in recoveries for the rest of PCPs, as it can clearly be observed in Figure S2. This is a logical feature, because the dispersive solvent can partially solubilize the extractant solvent. Worse precision was also observed when acetonitrile was utilized. Therefore, acetonitrile was not really required in the proposed VAEME method for the selected group of PCPs.

We also select a wide group of surfactants to carry out the study of the influence of surfactants in the VAEME performance, from a variety of ionic to nonionic surfactants. Among ionic surfactants, the cationic surfactant cetyltrimethylammonium bromide (CTAB), the anionic surfactant sodium dodecyl sulfate (SDS), and the ionic liquid-based surfactants: hexadecylpyridinium chloride ($C_{16}$PyCl) and 1-hexadecyl-3-butylimidazolium bromide ($C_{16}$MImBr) were studied. The nonionic surfactant tested was polyoxyethylene-10-lauryl ether ($C_{12}E_{10}$). In all cases, the tested concentration was close (but slightly lower) than their respective critical micelle concentration values. Figure 4 shows the results obtained. Clearly, the use of surfactants was not really successful in the improvement of the overall performance if compared to the neat VAEME method. For the UV filters BP and BP-3, it seems that CTAB slightly improves the extraction efficiency *versus* neat VAEME.

In this work, we decided not to use any surfactant neither dispersive solvent, because the simplified VAEME method was adequate to extract the group of PCPs selected.

### 3.6 Analysis of environmental water samples with the optimum VAEME-UHPLC-UV method

Several environmental water samples were analyzed with the optimized VAEME-UHPLC-UV method for the determination of PCPs. All samples considered were from the Island of Tenerife: two swimming pool waters (SP1 and SP2), two seawaters (SW1 and SW2), two wastewaters (WW1 and WW2) and one tap water (TW). All waters were sampled as described in Section 2.2., and analyzed by triplicate with the overall VAEME-UHPLC-UV method (Table 4). MPb was detected in 5 of the samples analyzed, and was quantified at 1.9 $\mu g \cdot L^{-1}$ in TW. Other PCPs were also quantified: BPb at 1.1 ± 0.3 $\mu g \cdot L^{-1}$ and Tr at 19.8 $\mu g \cdot L^{-1}$, in WW1 and WW2, respectively. BPb and *i*BPb were detected in TW, and *i*BPb was detected in SP1. Obvious caution with these results is advisable, because UV and not MS detection has been utilized in this work. MS is the detector of choice when unequivocal identification is pursued. It must be highlighted that in this work the solvent used for injection in the UHPLC is the LC mobile phase, and so the present VAEME-UHPLC-UV method is totally applicable as VAEME-UHPLC-MS method. In any case, these results are comparable with literature works. For example, Tr has been quantified at 0.041 $\mu g \cdot L^{-1}$ in effluents of wastewaters using IL-DLLME-LC-MS/MS[19], and at 0.1 $\mu g \cdot L^{-1}$ in influents of wastewaters using SBSE-UHPLC-MS/MS[23]. Other authors have quantified Tr at 2.08 $\mu g \cdot L^{-1}$ in domestic waters using DLLME-UHPLC-UV-Vis.[21]

Three representative water samples of different nature were also utilized to evaluate the matrix effect: SP2, SW1 and WW1. These samples were spiked at an intermediate concentration level of PCPs (12.5 $\mu g \cdot L^{-1}$), and then analyzed six times by the overall

method (intra-day). Table 4 also shows the performance of the method with these samples, in terms of relative recovery, intra-day precision, and extraction efficiency.

The average RR values obtained were 93.9 ± 13.1% for SP2, 87.8 ± 15.6% for SW1, and 67.4 ± 14.2% for WW1. Relative recoveries obtained for SP2 and SW1 are similar to those with deionized water. However, the matrix effect is clear in the wastewater sample, which can be justified by its high organic matter content.

The average extraction efficiencies were 75.6%, 71.5%, and 54.5% for SP2, SW1 and WW1, respectively. These values are comparable with those obtained with deionized water at the intermediate spiked level (82.0%) for swimming pool waters and seawaters, and again the matrix effect is clear for wastewaters.

# 4 Conclusions

A simplified vortex-assisted emulsification microextraction method combined with ultra-high performance liquid chromatographic UV detection has been applied for the first time for the determination of ten personal care products including seven parabens, two UV filters and one disinfectant, from environmental waters of different nature and complexity.

The main advantages of the present method include: short analysis time (~10 min for the VAEME procedure and ~12 min for the UHPLC), simplicity in the optimization and development, environmental friendliness (only 200 µL of extractant solvent), and adequate analytical performance even at the low spiked level (3.75 $\mu g \cdot L^{-1}$): in terms of relative recoveries (average value of 112%), enrichment factors (between ~20 and ~100), intra- and inter-day precision (below 10% as RSD), and extraction efficiency (average value of 82.7%).

Furthermore, the method only requires the utilization of trichloromethane as extractant solvent while applying vortex for 3 minutes to 8 mL of aqueous sample, and it does not require any dispersive solvent neither surfactant to help in the emulsification procedure.

## Acknowledgements

V.P. thanks the Spanish Ministry of Economy and Competitiveness (MINECO) for the Ramón y Cajal contract with the University of La Laguna (ULL) and the MINECO Project Ref. MAT2013-43101-R. J.H.A. and V.P. also thank funding from Fundación CajaCanarias project ref. SPDs-AGUA05. P.G.-H. thanks Fundación CajaCanarias project ref. SPDs-AGUA05 for her contract with ULL.

# References


1 W.W. Buchberger, *J. Chromatogr. A*, 2011, **1218**, 603–618.

2 M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, *Trends Anal. Chem*., 2011, **30**, 749−760.

3 R. Rodil, J.B. Quintana, P. López−Mahía, S. Muniategui−Lorenzo, D. Prada−Rodríguez, *Anal. Chem*., 2008, **80**, 1307−1315.

4 N. Negreira, I. Rodríguez, M. Ramil, E. Rubí, R. Cela., *Anal. Chim. Acta*, 2009, **654**, 162−170.

5 M.C. Pietrogrande, G. Basaglia, *Trends Anal. Chem*., 2007, **26**, 1086−1094.

6 S. Ortiz de García, G. Pinto−Pinto, P. García−Encina, R. Irusta−Mata, *Sci. Total Environ.*, 2013, **444**, 451−465.

7 M.M.P. Tsui, H.W. Leung, P.K.S. Lama, M.B. Murphy, *Water Res.*, 2014, **53**, 58−67.

8 A.M. Peck, *Anal. Bioanal. Chem*., 2006, **386**, 907−939.

9 M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, *J. Chromatogr. A*, 2009, **1216**, 6994−7000.

10 R.A. Yucuis, C.O. Stanier, K.C. Hornbuckle, *Chemosphere*, 2013, **92**, 905−910.

11 C. Cortada, L. Costa dos Reis, L. Vidal, J. Llorca, A. Canals, *Talanta*, 2013, **120**, 191−197.

12 M. López−Nogueroles, A. Chisvert, A. Salvador, A. Carretero, *Talanta*, 2011, **85**, 1990−1995.

13 L. Vallecillos, E. Pocurull, F. Borrull, *Talanta*, 2012, **99**, 824−832.

14 M.A. Mottaleb, S. Usenko, J.G. O'Donnell, A.J. Ramirez, B.W. Brooks, C.K. Chambliss, *J. Chromatogr. A*, 2009, **1216**, 815−823.

15 M.A. Farajzadeh, E.M. Khosrowshahi, P. Khorram, *J. Sep. Sci.*, 2013, **36**, 3571−3578.

16 J. Regueiro, M. Llompart, E. Psillakis, J.C. García−Monteagudo, C. Garcia−Jares, *Talanta*, 2009, **79**, 1387−1397.

17 M.C. Alcudia−León, R. Lucena, S. Cárdenas, M. Valcárcel, *Microchem. J.*, 2013, **110**, 643−648.

18 L. Vidal, A. Chisvert, A. Canals, A. Salvador, *Talanta*, 2010, **81**, 549−555.

19 R.S. Zhao, X. Wang, J. Sun, S.S. Wang, J.P. Yuan, X.K. Wang, *Anal. Bioanal. Chem*., 2010, **397**, 1627−1633.

20 C. Liao, S. Lee, H.B. Moon, N. Yamashita, K. Kannan, *Environ. Sci. Technol*., 2013, **47**, 10895−10902.

21 J.H. Guo, X.H. Li, X.L. Cao, Y. Li, X.Z. Wang, X.B. Xu, *J. Chromatogr. A*, 2009, **1216**, 3038−3043.

22 S. Montesdeoca−Esponda, Z. Sosa−Ferrera, J.J. Santana−Rodríguez, *J. Sep. Sci*., 2013, **36**, 781−788.

23 M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, *Anal. Bioanal. Chem*., 2010, **397**, 2833−2839.

24 B.R. Ramaswamy, J.W. Kim, T. Isobe, K.H. Chang, A. Amanoe, T.W. Miller, F.P. Siringan, S. Tanabe. *J. Hazard. Mater*., 2011, **192**, 1739−1745.

25 A. Gałuszka, Z. Migaszewski, J. Namieśnik, *Trends Anal. Chem*., 2013, **50**, 78–84.

26 M. Farré, S. Pérez, C. Gonçalves, M.F. Alpendurada, D. Barcelo, *Trends Anal. Chem.*, 2010, **29**, 1347–1362.

27 M. Rezaee, Y. Assadi, M.R. Millani, E. Aghaee, F. Ahmadi, S. Berijani, *J. Chromatogr. A*, 2006, **1116**, 1−9.

28 M.I. Leong, M.R. Fuh, S.D. Huang, *J. Chromatogr. A*, 2014, **1335**, 2–14.

29 H. Yan, H. Wang, *J. Chromatogr. A*, 2013, **1295**, 1−15.

30 F. Maya, B. Horstkotte, J.M. Estela, V. Cerdà, *Trends Anal. Chem.*, 2014, **59**, 1−8.

31 R.S. Zhao, X. Wang, J. Sun, C. Hu, X.K. Wang, *Microchim. Acta*, 2011, **174**, 145–151.

32 X. Xu, Z. Liu, X. Zhao, R. Su, Y. Zhang, J. Shi, Y. Zhao, L. Wu, Q. Ma, X. Zhou, H. Zhang, Z. Wang, *J. Sep. Sci*., 2013, **36**, 585–592.

33 V. Andruch, M. Burdel, L. Kocúrová, J. Šandrejová, I.S. Balogh, *Trends Anal. Chem.*, 2013, **49**, 1−19.

34 E. Yiantzi, E. Psillakis, K. Tyrovola, N. Kalogerakis, *Talanta*, 2010, **80**, 2057−2062.

35 S. Ozcan, *Clean−Soil Air Water*, 2010, **38**, 457−465.

36 T. Wu, W. Zhao, Z. Yang, H. Gao, Z. Zhou, *J. Sep. Sci*., 2013, **36**, 3918–3925.

37 Y. Lian, X. Qiu, Y. Yang, *Food Anal. Meth*., 2014, **7**, 636−644.

38 Y. Li, Y. Jiao, Y. Guo, Y. Yang, *Anal. Methods*, 2013, **5**, 5037−5043.

39 J.A. Oviedo, L.L. Fialho, J.A. Nóbrega, *Spectroc. Acta Pt. B−Atom. Spectr*., 2013, **86**, 142–145.

40 I.P. Román, A. Mastromichali, K. Tyrovola, A. Canals, E. Psillakis, *J. Chromatogr. A*, 2014, **1330**, 1−5.

41 C. Bosch–Ojeda, F. Sánchez–Rojas, *Chromatographia*, 2014, **77**, 745–754.

42 K. Seebunrueng, Y. Santaladchaiyakit, S. Srijaranai, *Chemosphere*, 2014, **103**, 51–58.

43 J. López−Darias, M. Germán−Hernández, V. Pino, A.M. Afonso, *Talanta*, 2010, **80**, 1611−1618.

44 L. Zhang, F. Chen, S. Liu, B. Chen, C. Pan, *J. Sep. Sci*., 2012, **35**, 2514−2519.

45 Z.H. Yang, Y.L. Lu, Y. Liu, T. Wu, Z.Q. Zhou, D.H. Liu, *J. Chromatogr. A*, 2011, **1218**, 7071−7077.

46 R.H. Li, D.H. Liu, Z.H. Yang, Z.Q. Zhou, P. Wang, *Electrophoresis*, 2012, **33**, 2176−2183.

47 Z.H. Yang, D.H. Liu, W.T. Zhao, T. Wu, Z.Q. Zhou, P. Wang, *J. Sep. Sci*., 2013, **36**, 916−922.

48 G. Leng, W. Chen, M. Zhang, F. Huang, Q. Cao, *J. Sep. Sci*., 2014, **37**, 684–690.

49 J. Vichapong, R. Burakham, S. Srijaranai, *Talanta*, 2013, **117**, 221–228.

50 Z.H. Yang, P. Wang, W.T. Zhao, Z.Q. Zhou, D.H. Liu, *J. Chromatogr. A*, 2013, **1300**, 58–63.

51 Y. Zhang, H.K. Lee, *J. Chromatogr. A*, 2013, **1274**, 28–35.

52 Y. Zhang, H.K. Lee, *J. Chromatogr. A*, 2012, **1259**, 25−31.

53 Q.Q. Baltazar, J. Chandawalla, K. Sawyer, J.L. Anderson, *Colloids Surf. A-Physicochem. Eng. Asp.*, 2007, **302**, 150–156.

54 E. Gracia−Lor, M. Martínez, J.V. Sancho, G. Peñuela, F. Hernández, *Talanta*, 2012, **99**, 1011−1023.

# Figure Captions

**Fig. 1** Average recoveries (%) only referred to the stage of evaporation/reconstitution for each PCPs studied, as a function of the solvent used in this step.

**Fig. 2** Effect of the extractant solvent volume on the overall extraction efficiency by VAEME–UHPLC–UV for the studied PCPs (n = 3), utilizing **A)** tetrachloroethylene, and **B)** trichloromethane. The remaining conditions of the method were described in the text.

**Fig. 3** Influence of the NaCl content (w/v) in the VAEME efficiency (n = 3) when using as extractant solvents: **A)** tetrachloroethylene, and **B)** trichloromethane. The remaining conditions of the method were described in the text.

**Fig. 4** Influence of different surfactants in the VAEME performance (n = 3). In all cases, the optimum conditions corresponding to the VAEME method using trichloromethane, with a spiked PCP concentration of 12.5 $\mu g \cdot L^{-1}$.

**Table 1**

Main physicochemical properties of the solvents initially considered as valid extractant solvents in VAEME.

| Solvent | Density at 20°C ($g \cdot cm^{-3}$) | Boiling point (°C) | Vapor pressure at 25°C (Pa) | Water solubility at 20°C ($g \cdot mL^{-1}$) | $logK_{OW}$ |
|---|---|---|---|---|---|
| Octanol | 0.823 | 194.7 | 15.20 | $3.0 \times 10^{-7}$ | 2.876 |
| Decanol | 0.828 | 227.8 | 1.97 | $3.7 \times 10^{-5}$ | 3.895 |
| Dichloromethane | 1.252 | 39.6 | $59.73 \times 10^{3}$ | $1.3 \times 10^{-2}$ | 1.405 |
| Trichloromethane | 1.500 | 61.2 | $26.67 \times 10^{3}$ | $8.0 \times 10^{-3}$ | 1.935 |
| Tetrachloroethylene | 1.653 | 119.1 | $2.57 \times 10^{3}$ | $1.5 \times 10^{-4}$ | 3.070 |

Data obtained from the SciFinder Scholar® 2014 database

**Table 2**

Quality analytical parameters of the calibrations for the overall VAEME-UHPLC-UV method using trichloromethane as extractant solvent.

| PCPs | (Slope ± $S_b$[a])×$10^{-3}$ | (Intercept ± $S_a$[b])× $10^{-3}$ | $S_{y/x}$×$10^{-3}$ | $R^2$ | LOD[c] (μg·$L^{-1}$) | LOQ[c] (μg·$L^{-1}$) |
|---|---|---|---|---|---|---|
| MPb | 7.6 ± 0.3 | 6 ± 4 | 7.08 | 0.9927 | 0.03 | 1.78 |
| EPb | 18.8 ± 0.6 | -9 ± 8 | 13.9 | 0.9944 | 0.58 | 0.87 |
| *i*PPb | 22.5 ± 0.5 | -6 ± 8 | 13.0 | 0.9966 | 0.45 | 0.93 |
| PPb | 23.4 ± 0.3 | -7 ± 5 | 8.17 | 0.9988 | 0.52 | 1.07 |
| *i*BPb | 19.4 ± 0.2 | -2 ± 2 | 3.96 | 0.9996 | 0.26 | 0.60 |
| BPb | 23.1 ± 0.2 | -5 ± 2 | 4.09 | 0.9997 | 0.35 | 0.69 |
| BzPb | 19.0 ± 0.2 | -1 ± 3 | 5.44 | 0.9992 | 0.36 | 1.13 |
| BP | 10.9 ± 0.3 | -9 ± 3 | 5.04 | 0.9962 | 1.33 | 2.57 |
| BP-3 | 15.4 ± 0.4 | -9 ± 6 | 9.76 | 0.9959 | 1.48 | 3.61 |
| Tr | 3.04 ± 0.05 | -1 ± 1 | 1.13 | 0.9986 | 1.65 | 3.49 |

[a]Error associated to slope
[b]Error associated to intercept
[c]LOD and LOQ calculated according to the ratio signal/noise as 3 and 10 times, respectively

**Table 3**

Analytical performance of the overall VAEME-UHPLC-UV method at two different spiked levels, in terms of intra-day precision, inter-day precision, extraction efficiency, relative recovery and enrichment factor.

| PCP | Spiked level: 3.75 µg·L$^{-1}$ | | | | | | Spiked level: 16.2 µg·L$^{-1}$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | RSD[a] intra-day (%) | RSD[b] inter-day (%) | RR[c] (%) | $E_F$[d] | $E_F$'[e] | $E_R$[f] (%) | RSD[a] intra-day (%) | RSD[b] inter-day (%) | RR[c] (%) | $E_F$[d] | $E_F$'[e] | $E_R$[f] (%) |
| MPb | 7.4 | 7.7 | 114 | 30.3 | 21.8 | 37.9 | 18 | 7.0 | 98.9 | 22.8 | 21.8 | 28.5 |
| EPb | 6.8 | 9.0 | 118 | 57.0 | 52.1 | 71.2 | 11 | 6.8 | 103 | 52.7 | 52.1 | 65.9 |
| *i*PPb | 1.0 | 5.9 | 114 | 76.0 | 69.5 | 95.0 | 9.5 | 5.2 | 103 | 70.7 | 69.5 | 88.4 |
| PPb | 2.7 | 5.6 | 115 | 70.7 | 66.4 | 88.4 | 6.8 | 4.7 | 104 | 67.9 | 66.4 | 84.9 |
| *i*BPb | 1.4 | 4.9 | 115 | 73.9 | 64.5 | 92.4 | 5.7 | 4.4 | 105 | 67.5 | 64.5 | 84.4 |
| BPb | 2.1 | 5.0 | 116 | 78.3 | 72.0 | 97.9 | 5.2 | 4.5 | 106 | 75.0 | 72.0 | 93.8 |
| BzPb | 2.4 | 5.2 | 113 | 85.9 | 74.1 | 107 | 4.5 | 4.5 | 107 | 79.9 | 74.1 | 99.9 |
| BP | 10 | 10 | 87.6 | 28.1 | 32.7 | 35.1 | 25 | 26 | 61.7 | 19.7 | 32.7 | 24.7 |
| BP-3 | 6.4 | 4.8 | 114 | 99.4 | 93.4 | 124 | 6.7 | 5.6 | 101 | 92.4 | 93.4 | 115 |
| Tr | 5.6 | 8.0 | 114 | 61.8 | 61.4 | 77.3 | 6.7 | 6.9 | 104 | 61.7 | 61.4 | 77.1 |

[a]Relative standard deviation, intra-day (n = 3)
[b]Relative standard deviation, inter-day (n = 9)
[c]Relative recovery
[d]Enrichment factor calculated as concentrations ratio
[e]Enrichment factor calculated as slopes ratio
[f]Extraction efficiency

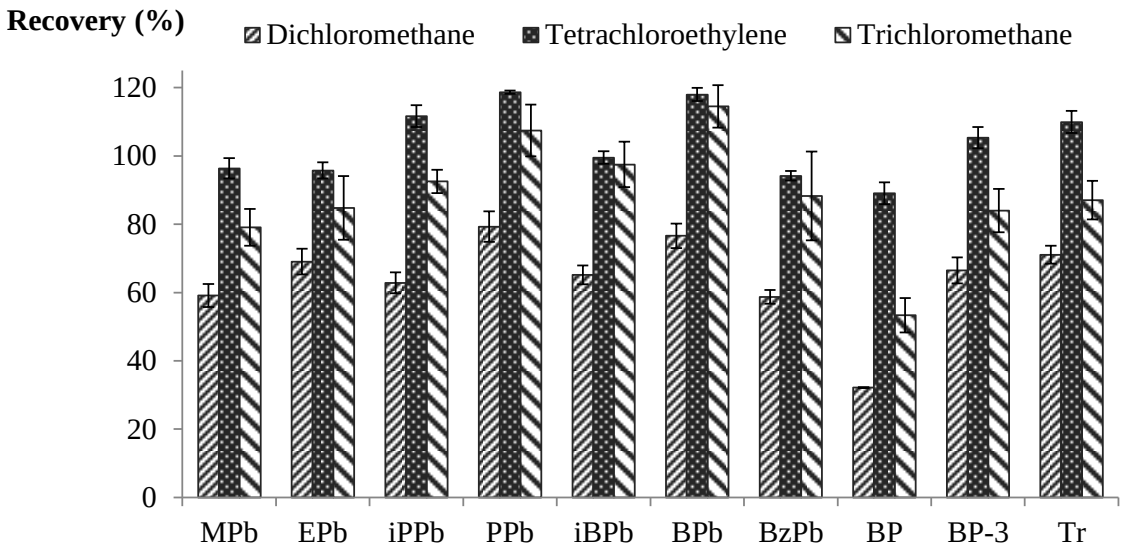
**Table 4**

Analysis of surface and wastewater samples using the overall optimized procedure.

| PCPs | SP1 | SP2 | | | SW1 | | | SW2 | WW1 | | | WW2 | TP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Level found ($\mu g \cdot L^{-1}$) | Level found ($\mu g \cdot L^{-1}$) | Spiked level: 12.5 $\mu g \cdot L^{-1}$ RR[a] (%) | $E_R$[b] (%) | Level found ($\mu g \cdot L^{-1}$) | Spiked level: 12.5 $\mu g \cdot L^{-1}$ RR[a] (%) | $E_R$[b] (%) | Level found ($\mu g \cdot L^{-1}$) | Level found ($\mu g \cdot L^{-1}$) | Spiked level: 12.5 $\mu g \cdot L^{-1}$ RR[a] (%) | $E_R$[b] (%) | Level found ($\mu g \cdot L^{-1}$) | Level found ($\mu g \cdot L^{-1}$) |
| MPb | ~1.0<LOQ | ~1.3<LOQ | 66.6 | 18.6 | ~0.5<LOQ | 75.2 | 22.5 | ~0.8<LOQ | ~0.4<LOQ | 53.4 | 16.6 | ~0.8<LOQ | 1.9 ± 0.1[c] |
| EPb | n.d. | n.d. | 89.1 | 51.5 | n.d. | 86.5 | 54.6 | n.d. | n.d. | 57.0 | 35.4 | n.d. | n.d. |
| *i*PPb | n.d. | n.d. | 90.2 | 77.0 | n.d. | 100 | 85.9 | n.d. | n.d. | 65.7 | 55.8 | n.d. | n.d. |
| PPb | n.d. | n.d. | 97.2 | 78.6 | n.d. | 103 | 83.7 | n.d. | n.d. | 67.3 | 53.8 | n.d. | n.d. |
| *i*BPb | ~0.4<LOQ | n.d. | 95.5 | 77.0 | n.d. | 102 | 82.0 | n.d. | n.d. | 76.9 | 62.0 | n.d. | ~0.3<LOQ |
| BPb | n.d. | n.d. | 106 | 93.4 | n.d. | 111 | 97.7 | n.d. | 1.1 ± 0.3[c] | 91.3 | 80.2 | n.d. | ~0.4<LOQ |
| BzPb | n.d. | n.d. | 98.8 | 92.4 | n.d. | 99.1 | 92.6 | n.d. | n.d. | 74.4 | 69.8 | n.d. | n.d. |
| BP | ~1.7<LOQ | ~1.4<LOQ | 120 | 48.8 | n.d. | 57.0 | 20.1 | n.d. | n.d. | 105 | 29.4 | n.d. | n.d. |
| BP-3 | n.d. | n.d. | 94.8 | 108 | n.d. | 95.9 | 109 | n.d. | n.d. | 75.8 | 85.8 | n.d. | n.d. |
| Tr | n.d. | n.d. | 65.0 | 46.7 | n.d. | 72.4 | 52.4 | n.d. | n.d. | 57.6 | 41.1 | 19.8 ± 0.2[c] | n.d. |

[a]Relative recovery
[b]Extractive efficiency
[c]Standard deviation (n = 3)
n.d.: non-detected

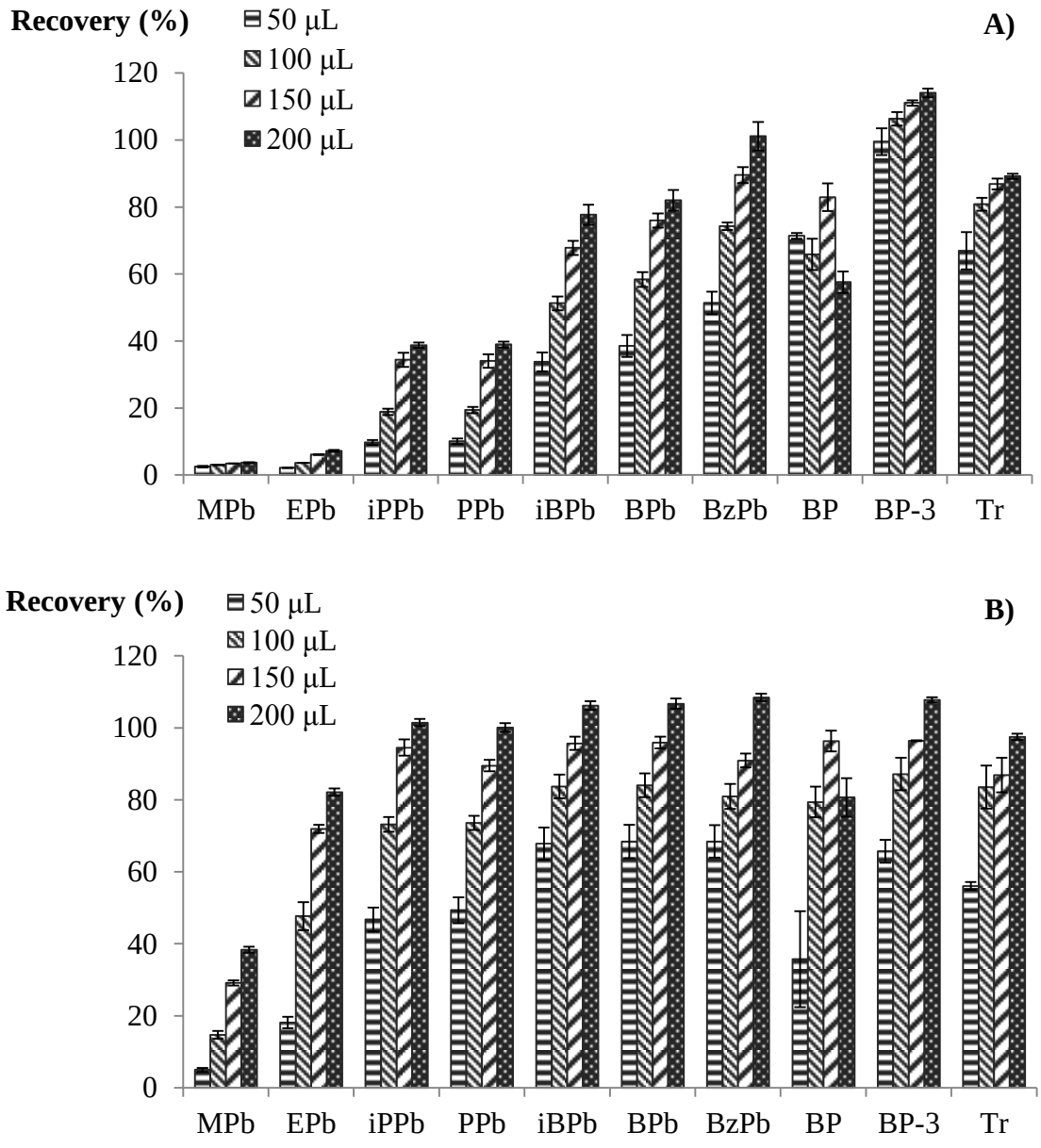
593 Figure 1




594

595

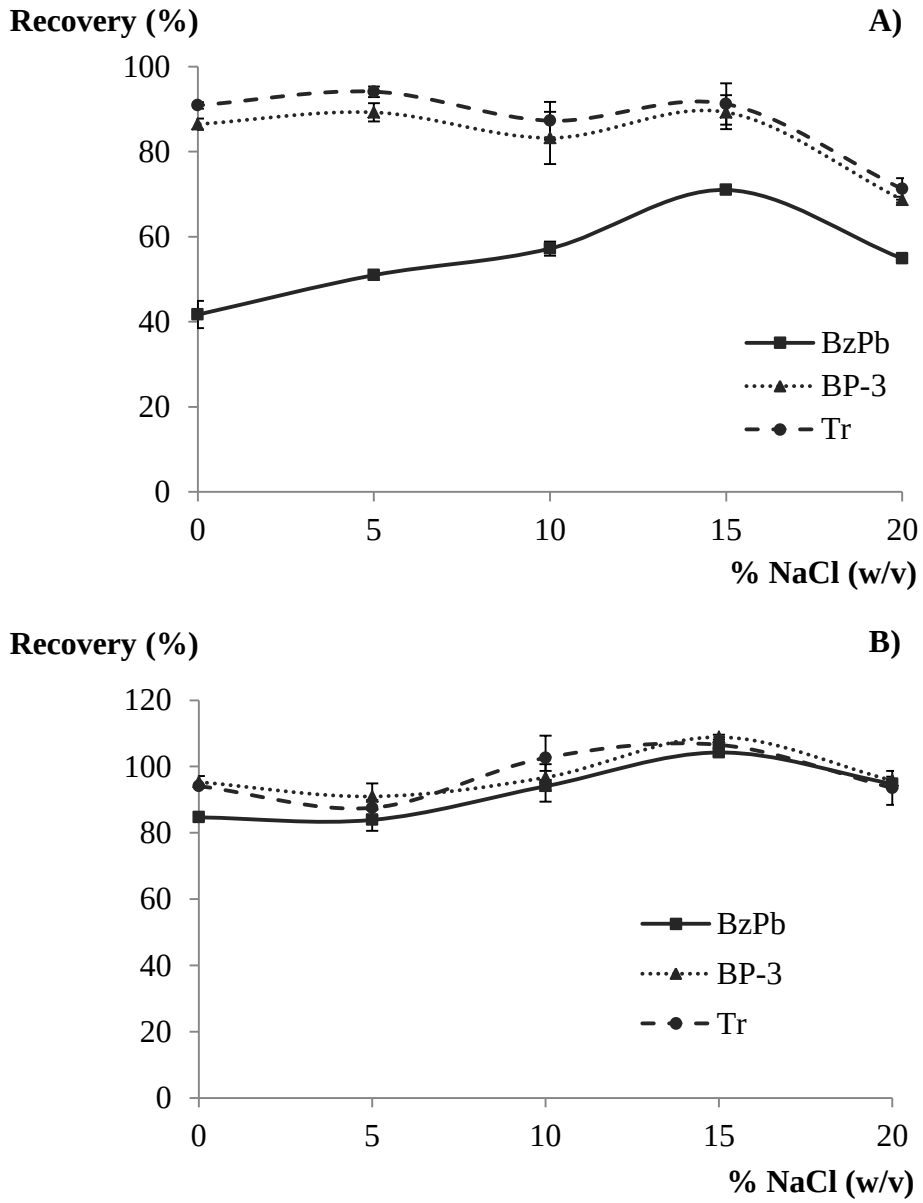
596 Figure 2




597

598

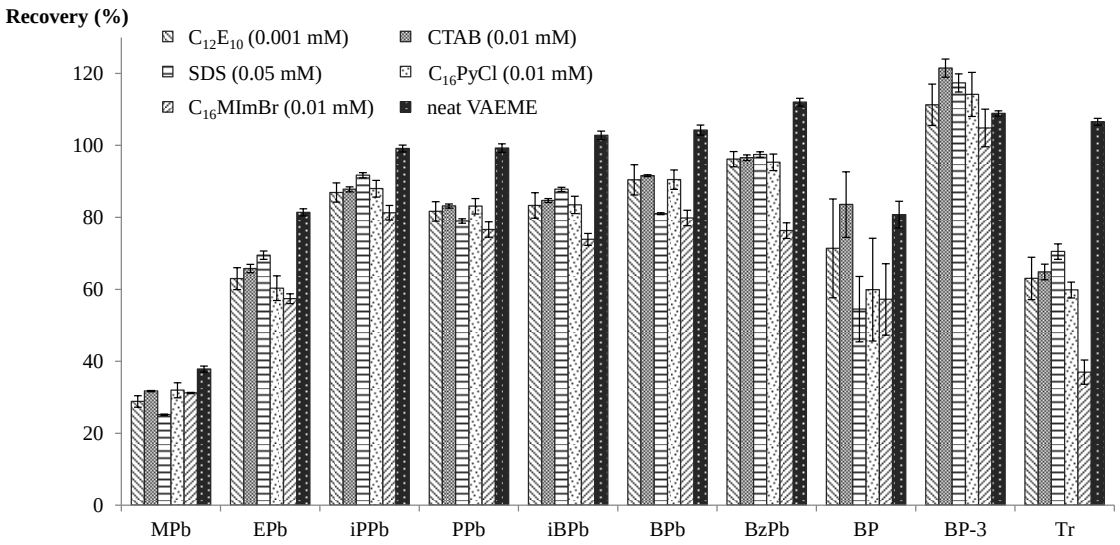
599 Figure 3




600

601

602 Figure 4




603