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Improvements on bar adsorptive microextraction (BAμE) technique–Application for the determination of insecticide repellents in environmental water matrices

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ABSTRACT

Bar adsorptive microextraction combined with micro-liquid desorption followed by large volume injection-gas chromatography–mass spectrometry operating in the selected-ion monitoring acquisition mode (BAμE-μLD/LVI-GC–MS(SIM)), is proposed for the determination of trace levels of three insecticide repellents (*N*,*N*-diethyl-meta-toluamide (DEET), *cis* and *trans* permethrin (PERM)) in environmental water matrices. By comparing different sorbent coatings (five activated carbons and six polymers) through BAμE, an activated carbon (AC2) proved to be the best compromise between selectivity and efficiency, even against polydimethylsiloxane through stir bar sorptive extraction. The novel improvement proposed on the back-extraction stage performed in a single step, by reducing the desorption solvent volume at the microliter level, demonstrated remarkable performance turning possible to save time, making easier the practical manipulation and more environmentally friendly. Assays performed by BAμE(AC2)-μLD/LVI-GC–MS(SIM) on 25 mL of ultrapure water samples spiked at the 1.0 μg/L level, yielded recoveries ranging from 73.8 ± 8.8% (*trans*-PERM) to 96.4 ± 9.9% (DEET), under optimised experimental conditions. The analytical performance showed convenient detection limits (8–20 ng/L) and good linear dynamic ranges (0.04–4.0 μg/L) with suitable determination coefficients ($r^2 > 0.9963$, DEET). Excellent repeatability were also achieved through intraday (RSD < 14.9%) and interday (RSD < 11.9%) experiments. The novel improvement on downsizing the BAμE device to half-size proved to be either a promising option in forthcoming to reduce still more the desorption solvent volume without losing microextraction efficiency. By using the standard addition methodology, the application of the present analytical approach on tap, ground, river, swimming-pool and estuary water samples revealed good sensitivity at trace level and absence of matrix effects.

1. Introduction

By definition, a personal insect repellent is a substance that after applied to skin or clothes, deters insects from biting or disturbing a human. Ancient human knew about the repellent properties of some plants, and therefore used the same to protect against insects bites, which happens still today in rural communities. For instance, to avoid the transmission of malaria or Lyme disease, the development and application of these repellents have increased significantly over past years. *N*,*N*-diethyl-meta-toluamide (DEET) is one of the most used personal insecticide repellent nowadays, which was developed by US Army to be used by militaries and after to general population. It is considered a chemical with moderate toxicity to humans, but its high use can lead to several toxic effects, such as seizure, dermatitis, acute manic psychosis, among others [1–3]. One of the main open doors of DEET to the environment is through the release via wastewater treatment plants, going after direct to the aquatic ecosystem, but also by recreational activities [2,4]. Permethrin (PERM) developed in 1973, is another insecticide commonly used as repellent that belongs to the pyrethroids group, which is a synthetic derivative of pyrethrins, the natural constituents of flowers. PERM is an active broad-spectrum insecticide, applied in several areas such as textile and carpet industries, wood preservative, veterinary, agriculture, public health and household applications. Therefore, several doors are continuously open to the aquatic system, such as irrigation, laundry, rain and many other ways [5–8]. Both *cis* and *trans* PERM isomers present insecticide activity, although the former form displays the strongest effect, and moreover, the toxicity must be evaluated through the ratio of both isomers [6]. This substance present low toxicity to mammal, but can be dangerously toxic to fish, cats, and have teratogenicity,

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carcinogenicity and mutagenicity harm effects [6,8]. Thus, the data about the concentration and fate of these pollutants in the environment is urgently needed, and analytical methods for a rapid, sensitive and selective determination of these compounds in water matrices are required. Due to the trace concentration levels (parts-per-billion, μg/L) of insecticides usually found in contaminated water matrices, state-of-the-art analytical methodologies for the determination of these compounds are mainly based on enrichment procedures prior to chromatographic techniques, such as gas chromatography (GC), high-performance liquid chromatography or even coupled to mass spectrometry (MS) [5,7,9–15]. Nowadays, the sorption-based methods are used at large, in which solid-phase extraction [7,10,13,15], solid phase microextraction (SPME) [11] and later, stir bar sorptive extraction (SBSE) [5,9,14] has been proposed as sample enrichment techniques for trace level analysis of insecticide repellents in water matrices. More recently, our group has introduced a novel static microextraction technique, *i.e.* bar adsorptive microextraction (BAμE), which uses nanostructured sorbents that is a remarkable alternative for trace analysis of medium-polar to polar compounds in aqueous media [16]. This new analytical methodology, which operates under the floating sampling technology, present also a great advantage comparatively to other sorption-based approaches (*e.g.* SBSE) [17], once allows to tune the most convenient sorbent phase (*e.g.* activated carbons (ACs), polymers (Ps), *etc.*) for each particular type of targets or classes of compounds. Besides the high performance and effectiveness demonstrated in many applications [18–23], this microextraction approach has been proposed by using a convenient back-extraction stage for combination with the instrumental system, which involves two steps; a conventional liquid desorption (LD) step using a suitable solvent volume (*ca.* 1.5 mL) under sonification; and subsequently, a solvent switch step, *i.e.* solvent evaporation until dryness and re-dissolution into another solvent or eluent more compatible with the instrumental sample injection system. In the analytical point of view, this is the limiting-stage once the desorption solvent volume, as well as, the solvent switch step, makes in many cases the back-extraction stage not environmentally friendly, often cumbersome and time consuming. In this work, we propose to improve the back-extraction stage for BAμE in a single step by reducing the volume of the desorption solvent in several orders of magnitude to the microliter level, *i.e.* micro-liquid desorption (μLD), and eliminating the solvent switch step. These improvements aim to turn possible saving time, making easier the practical manipulation and a more environmentally friendly approach. To evaluate the performance of these novel improvements on BAμE-μLD, three insecticide repellents (DEET, *cis* and *trans* PERM) will be used as model compounds in water matrices, prior to large volume injection-gas chromatography coupled to mass spectrometry operating in selected-ion monitoring acquisition mode (LVI-GC–MS(SIM)). The optimization of this novel analytical approach, including the evaluation of the selectivity, interactions mechanism and equilibrium kinetics of the sorbent phases (five ACs and six Ps) tested, as well as, the influence of several experimental parameters and the downsizing of the analytical device, is fully discussed. The validation and the application of the optimised methodology for the determination of trace levels of the insecticide repellents in real matrices are also addressed.

2. Experimental

2.1. Standards and samples

In this study all standards were neat certified standards chemicals. DEET (98.0%) and permethrin (PERM, *cis*- and *trans*- isomers mixture, 94.4%) were supplied by Acros Organics (USA) and Riedel-de-Haën (Seelze, Germany), respectively. The chemical structures of all three repellents are depicted in Fig. 1. The solvents used were HPLC-grade methanol (MeOH, 99.8%) and acetonitrile (ACN, 99.8%) obtained from Fisher (UK), and hexane (*n*-C6, 99%) from Panreac (Spain). Sodium chloride was supplied from Merck (99.5%; Germany), sodium hydroxide pellets was obtained from AnalaR (98.0%; BDH chemicals, UK) and hydrochloric acid 37% was provided from Panreac (Spain). Ultra-pure water was obtained from the Milli-Q water purification systems (USA). The polymeric phases used were modified pyrrolidone (P1; particle size: 33 μm; pore size: 85 Å; surface area: 800 m^2/g; pH stability: 1–14; USA), styrene-divinylbenzene (P2; particle size: 100 μm; pore size: 260 Å; surface area: 500 m^2/g; pH stability: 1–14; USA) and, ciano (P3; particle size: 55 μm, pore size: 70 Å, surface area: 500 m^2/g; USA), furnished by Tecnocroma (Portugal). The ionic polymer sorbents with anion exchange/reversed-phase (P4; particle size: 30 μm; pore size: 80 Å; surface area: 830 m^2/g; pH stability: 0–14) and cation exchange/reversed-phase characteristics (P5; particle size: 60 μm; pore size: 80 Å; surface area: 830 m^2/g; pH stability: 0–14) were supplied by Via Athena (Portugal). The stir bars having polydimethylsiloxane (PDMS, 126 μL; P6) were supplied from Gerstel (Germany).The ACs used were AC1 (pH_{PZC}: 6.4; surface area: 1400 m^2/g), AC2 (pH_{PZC}: 2.2; surface area: 1400 m^2/g), AC3 (pH_{PZC}: 7.5; surface area: 1100 m^2/g), AC4 (pH_{PZC}: 8.5; surface area: 1500 m^2/g) and AC5 (pH_{PZC}: 8.4; surface area: 900 m^2/g), provided by Salmon & Cia (Portugal). Stock solutions of individual insecticide repellents (1000 mg/L) used for the working standard mixture were prepared in ACN, stored at −20 °C and renewed every month. For instrumental calibration, standard mixtures were prepared in ACN by appropriate dilution of the previous stock solutions. The water samples were collected in the metropolitan area of Lisbon (Portugal); the tap water was collected from our lab, the ground water was taken in a well, the estuarine water was collected from Tagus estuary, the river water was collected from Alviela river and the swimming-pool water was collected from a public swimming-pool. Solutions of sodium hydroxide (0.1 M) and hydrochloric acid (5%) were used for pH adjustments.

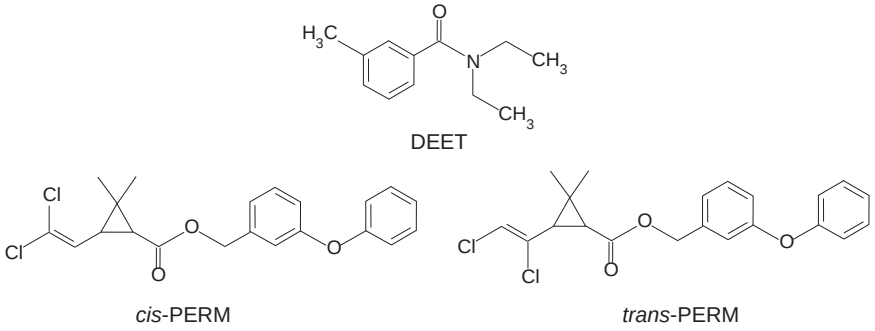

Fig. 1. Chemical structures of the insecticides used in this study.

2.2. Experimental assays

The pH of the point of zero charge (pH_{PZC}) was previously determined according to several authors [24], and is defined by the pH of the plateau equilibrium curve against the solid weight fraction, using a Metrohm 744 pH metre with a combined glass electrode (Switzerland). The BAμE devices (15 mm in length and 3 mm o.d.; 2–5 mg in sorbent mass; Fig. 2(a1)) were lab-made prepared according to previous work [16]. For the Ps phases, each BAμE device had an average sorbent weight of 2.5 ± 0.2 mg (P1), 3.9 ± 0.3 mg (P2), 4.5 ± 0.3 mg (P3), 1.6 ± 0.1 mg (P4) and 2.0 ± 0.2 mg (P5). For the ACs phases, each BAμE bar has an average sorbent weight of 1.8 ± 0.1 mg (AC1), 1.7 ± 0.3 mg (AC2), 2.8 ± 0.1 mg (AC3), 3.2 ± 0.3 mg (AC4) and 1.9 ± 0.2 mg (AC5). The BAμE devices were previously cleaned with ultrapure water before use. The half-size BAμE (½BAμE) devices were also lab-made prepared according to previous work [16], having similar bar-shaped geometry (7.5 mm in length and 3 mm o.d.; ≈ 0.9 mg in AC2 mass; Fig. 2(a2)). Typical assays were performed in a sampling flask having 25 mL of ultrapure water spiked with an appropriate volume of a working standard mixture to get a concentration of 1.0 μg/L, followed by the introduction of the SBSE or BAμE device, previously coated with powdered sorbent, and a conventional Teflon stir bar performed in a multipoint agitation plate (Variomag, Germany) at room temperature. In a first approach, several AC and P sorbents were tested in order to evaluate the selectivity that reaches the best recovery yields performed under standard experimental conditions; extraction: 3 h (1000 rpm), 1.0 μg/L, pH 5.5; back-extraction: 200 μL ACN during 30 min under ultrasonic treatment. After selecting the sorbent that evidenced the higher analytical response, and in order to achieve the best BAμE efficiency process, systematic studies were performed in triplicate for the optimization of several parameters such as equilibrium time (1, 2, 3 and 16 h), pH (2.0, 5.5, 8.0 and 11.0), stirring speed (750, 1000 and 1250 rpm), organic modifiers (MeOH; 5, 10 and 15%, v/v) and ionic strength (NaCl; 5, 10 and 15%, w/v). After extraction, the devices were removed from the samples with clean tweezes and placed into inserts containing 200 μL of the stripping solvent, inside a 2 mL vial, ensuring their total immersion prior to ultrasonic treatment (Branson 3510; Switzerland) at room temperature. For μLD, *n*-C6, ACN, MeOH and mixtures of ACN/MeOH (1:1, v/v) were the stripping solvents tested under ultrasonic treatment times at 15, 30, 45 and 60 min. The vials were then sealed and placed on the auto-sampler for LVI-GC–MS (SIM) analysis. For method validation experiments, 25 mL of ultrapure water were spiked with appropriate volume of working standard mixture to reach the desired concentrations and then, the assays were performed under optimised experimental conditions. The application to real matrices was performed in triplicate using 25 mL of each sample, through the standard addition method (SAM) and operating under optimised experimental conditions. Blank assays were also performed using the procedure above without spiking.

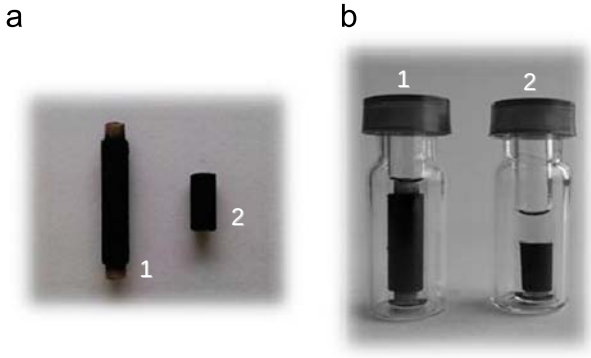

Fig. 2. Images showing the bar-shaped geometry of the BAμE (1) and ½BAμE (2) devices (a) and during the μLD process (b).

2.3. Instrumental set-up

LVI-GC–MS(SIM) analysis was performed using an Agilent 6890 Series gas chromatograph equipped with an Agilent 7683 automatic liquid sampler coupled to an Agilent 5973 N mass selective detector (Agilent Technologies, Little Falls, DE, USA). A programmed temperature vaporisation injector with a liner filled with glass wool and liquid nitrogen used as inlet cooling was used operating under solvent-vent mode injection (vent time 0.30 min; flow 100 mL/min; pressure 0 psig; purge flow 60 mL/min at 2 min). The inlet temperature was programmed from 20 °C (0.35 min) to 320 °C (3 min isothermal) at a rate of 600 °C/min and subsequently decreased to 200 °C (held until end) at a rate of 20 °C/min. The injection volume and speed were 20 μL and 100 μL/min, respectively. GC analysis was performed on a TRB-5MS (30 m × 0.25 mm ID, 0.25 μm film thickness) capillary column (5% diphenyl, 95% dimethyl polysiloxane; Teknokroma, Spain) with helium as carrier gas maintained in the constant-pressure mode (53 cm/s). The oven temperature was programmed from 100 °C (held 1 min) to 240 °C at 40 °C/min, and then at 4 °C/min to 290 °C (held for 1 min) in 18.00 min of total running time. The transfer line, ion source, and quadrupole analyzer temperatures were set at 280, 230, and 150 °C, respectively, and a solvent delay of 2 min was selected. In full-scan mode acquisition, electronic ionization mass spectra in the range 35–550 Da were recorded at 70 eV electron energy with an ionization current of 34.6 μA. In selected ion monitoring (SIM) mode acquisition, two groups of target ions were monitored at different time windows defined by the corresponding retention times (see Table 1), maintaining a dwell time of 100 ms. For quantification, three qualifier ions were chosen for each target analyte, according to the characteristic features of the mass spectra obtained in full-scan mode and by comparison with the Wiley's library spectral data bank (G1035B; Rev D.02.00; Agilent Technologies). Data recording and instrument control were performed by the MSD ChemStation software (G1701CA; Version C.00.00; Agilent Technologies). The recovery data of all assays performed were calculated through the comparison of the average peak areas of the extracted analytes with standard controls having the same concentration.

3. Results and discussion

3.1. Evaluation of the LVI-GC–MS(SIM) performance

In the present work, three insect repellents (DEET, *cis*-PERM and *trans*-PERM) were selected as model compounds (Fig. 1) having different chemical properties (Table 1). The first step was the evaluation of the mass spectral fragmentation pattern of each insecticide through the analysis of a standard mixture by GC–MS, operating in the full-scan mode acquisition. Based on the characteristic features of the spectral data, target base peaks and qualifier ions were chosen (Table 1) to achieve high selectivity and sensitivity for operating in the SIM mode acquisition, according to previous works [5,10,14,25]. By monitoring the selected ions, high response and symmetrical peak shape could be achieved in suitable analytical time (< 14 min), under convenient chromatographic conditions. To increase sensitivity, in particular for real sample analysis, large-volume injection (LVI) operating in the solvent vent mode was adopted during GC–MS(SIM) analysis. The injection was set at 20 μL since larger sample volumes led to high solvent background and, therefore, a lower signal-to-noise (S/N) ratio at trace level [23]. Instrumental calibration was subsequently performed with standard mixtures ranging from 1.0 to 500.0 μg/L through the external standard approach. The data obtained,

Table 1
Chemical class, octanol–water partition coefficients, ions, retention time, LODs, LOQs, linear dynamic ranges and determination coefficients, for the three insecticides obtained by LVI-GC–MS(SIM), under optimised instrumental conditions.

Insecticides	Class	log $K_{O/W}$[a]	Ions[b] (*m/z*)	RT (min)	LODs[c] (μg/L)	LOQs[d] (μg/L)	Linear range (μg/L)	r^2
DEET	*N,N*-dialkylamide	2.26	190, 119, 91	4.0	0.20	0.66	1.0–500.0	0.9969
cis-PERM	Pirethroid	7.43	283, 263, 127	11.6	0.20	0.66	1.0–500.0	0.9977
trans-PERM	Pirethroid	7.43	283, 263, 127	11.8	0.20	0.66	1.0–500.0	0.9972

[a] US EPA (2011) Estimation Programs Interface Suite™ for Microsoft® Windows, v. 4.1. United States Environmental Protection Agency, Washington, DC.
[b] Quantification (underlined) and qualifier ions.
[c] LODs at S/N=3.
[d] LOQs at S/N=10.

showed good linearity using the corresponding target ion abundances, where suitable determination coefficients ($r^2 > 0.9969$) were achieved. Instrumental sensitivity was also checked through the limits of detection (LODs) and quantification (LOQs), obtained by the injection of diluted standard mixtures of the target insecticides, and calculated with S/N of 3/1 and 10/1, where 0.20 μg/L and 0.66 μg/L were achieved, respectively. Moreover, during instrumental development, the precision expressed as relative standard deviations (RSD) were below 18.8% (*trans*-PERM). Carry-over was not observed after injections of blank runs, in which the background was always below the LODs. Table 1 summarizes some chemical properties as well as the instrumental data obtained by LVI-GC–MS(SIM) for the three insecticide repellents under study.

3.2. Evaluation of the BAμE-μLD efficiency

In this section, several experimental parameters that can affect the BAμE-μLD efficiency of the target compounds were evaluated, including the novel improvements developed. Therefore, the optimization was carried out in order to obtain maximum performance for the implementation of the proposed methodology. Hence, systematic studies were performed in ultrapure water samples spiked with the insecticides at the 1.0 μg/L level, involving the selection of the sorbent phase, equilibrium time, agitation speed, pH, polarity and ionic strength for extraction, as well as, solvent type, volume and desorption time for back-extraction, using a univariate optimization strategy according to previous works [18–23].

3.2.1. Selection and characterization of the nanostructured coatings

For the present study, we start to choose several nanostructured coatings having very different physico-chemical properties to be tested as sorbent phases by BAμE, according to previous reports [18–23,26]. Thus, six P (P1–P6) and five AC (AC1–AC5) coatings were tested as sorbent phases for the microextraction of the insecticide model compounds in aqueous media. The P coatings selected for the present work were constituted by modified pyrrolidone (P1), styrene-divinylbenzene (P2), ciano (P3), anion exchange/reversed-phase (P4), cation exchange/reversed-phase (P5) and polydimethylsiloxane (P6) phases, where the latter was applied through the well-established SBSE in order to compare also the performance of this new technique. The Ps are characterized to have particle sizes in between 30 and 100 μm, pore sizes ranging from 70 and 260 Å and surface areas among 500 and 830 m^2/g, presenting also a large range of pH stability (0 − 14). On the other hand, the solid coatings selected (AC1, AC2, AC3, AC4 and AC5) present surface areas ranging from 900 to 1500 m^2/g and pH_{PZC} values of 6.4, 2.2, 7.5, 8.5 and 8.4, respectively, presenting from acidic to basic characteristics. Therefore, from the data obtained, the tested ACs presented very different surface chemistry properties that can be crucial for the microextraction selectivity of the insecticide repellents under study. It must be point out that the three compounds present polar to non-polar (log $K_{O/W}$: 2.26 and 7.43) characteristics (Table 1), where DEET has weak basic behaviour and, PERM isomers, besides very stable do not suffer ionization. Even so, these properties can have a great influence on the type of interaction mechanisms involved with the selected sorbent phases during the microextraction process. Furthermore, DEET and PERM molecules present very different molecular geometric (Fig. 1), in which the former has smaller size and a more pronounced spherical shape.

3.2.2. Selectivity of the sorbent phases

Preliminary assays were performed in order to achieve the best selectivity of the different sorbent coatings tested, according to previous works [26]. Fig. 3 compares the selectivity obtained by using different phases, *i.e.* ACs (AC1–AC5; Fig. 3(a)) and Ps (P1–P6; Fig. 3(b)), as well as extraction techniques (BAμE and SBSE), for the microextraction of the insecticide repellents from ultrapure water matrices, under standard experimental conditions (extraction: 3 h (1000 rpm), 25 mL (1.0 μg/L), pH 5.5; back-extraction: ACN (200 μL), 30 min under ultrasonic treatment). Fig. 3(a) depicts good average recoveries promoted by all ACs for DEET (>60%), while AC1 and AC2 are the most selective (>25%) for *cis* and *trans* PERM, under similar experimental conditions. In spite of the textural adsorptive properties of all nanostructured sorbents used, which includes the surface area, particle size and pore dimensions, the interactions between the ACs and the insecticide molecules seems to be predominantly influenced by electrostatic and/or dispersive interactions. Therefore, once the pH_{PZC} can have a strong influence in almost all the retentions mechanism, the overall interactions are definitely conditioned by the pH_{PZC}, through the acidic or basic characteristics of the surface area of the materials involved. The main contribution for the acid/base nature of ACs, depends mostly on the heteroatoms occurrence at the net of the solid surface, such as oxygen, hydrogen and nitrogen, which will influence the adsorptive capacity of the ACs in liquid phase. For a pH sample solution (pH_{Sample}) equal to the pH_{PZC}, all positive and negative sites are present in the same number, and the resultant net charge of the solid surface is zero. Like this, if $pH_{Sample} < pH_{PZC}$, the net charge of the solid surface become positive, and negative if $pH_{Sample} > pH_{PZC}$ [19,27,28]. At the particular pH 5.5, the ACs surface charge ranges from negative (AC2) to almost neutral (AC1 and AC3) and positive (AC4 and AC5), which play an important role on the interactions with the molecules involved, which are neutral at that value. Therefore, the data demonstrate that the AC1 (pH_{PZC}: 6.4) and AC2 (pH_{PZC}: 2.2) interactions with the *cis* and *trans* PERM molecules is particularly influenced through the acidic character, where can be concluded that the higher is the acidity of the sorbent phase the better is the adsorption phenomena. Nevertheless, despite the acidic characteristics of ACs be critical for the interaction with *cis* and *trans* PERM molecules, it is negligible for DEET and therefore, the retention seems to be predominantly effective through electrostatic and dispersive interactions, respectively. Fig. 3(b) shows the profile obtained with the polymeric coatings, where can

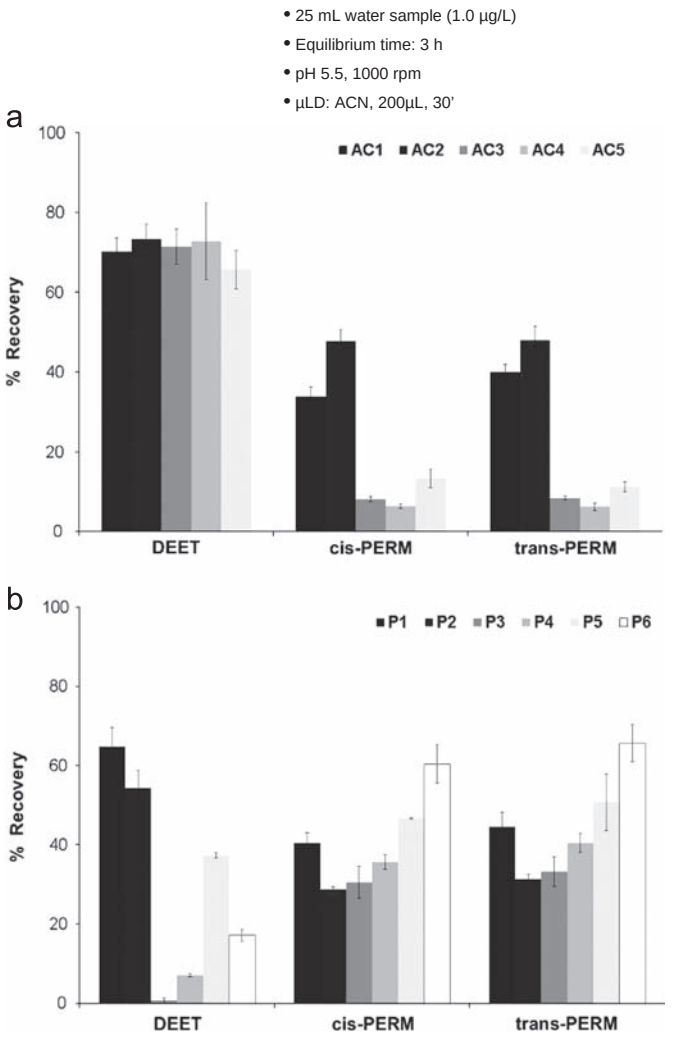

Fig. 3. Average recovery yields obtained by BAµE using different ACs (a) and Ps (b) phases for the three insecticides in aqueous matrices, using standard experimental conditions. (Extraction: 25 mL water sample (1 µg/L, pH 5.5), 3 h, 1000 rpm; Back-extraction: ACN (200 µL), 30′ under ultrasonic treatment).

be observed that both modified pyrrolidone (P1) and styrene-divinylbenzene (P2) phases seems to promoted much stronger chemical interactions and therefore, much better selectivity for DEET, whereas for PERM isomers, P6 gave the best efficiency yield (> 60%). It is well known that SBSE(PDMS) is largely indicated for nonpolar compounds (log $K_{O/W} \geq 3$), where the neutral molecules promote hydrophobic interactions (*i.e.* Van-der-Waals forces), the main retention mechanism of the silicone-type sorbents [17,26]. Therefore, PERM isomers were favoured with a recovery above 60% once both compounds present nonpolar characteristics (log $K_{O/W} = 7.43$). On contrary, DEET has lower efficiency (< 20%) since present a polar (log $K_{O/W} = 2.26$) behaviour. Moreover, the performance of the BAµE technique that operates under the floating sampling technology do not present any disadvantage when compared to SBSE, which works through the immersion sampling mode [16,26]. It is also notice that although ciano (P3), anion exchange/reversed-phase (P4) and cation exchange/reversed-phase (P5) phases present some selectivity for both PERM isomers (25–50%), for DEET in particular, the latter and the former sorbents are the more and less favourable, respectively. Several mechanisms of retention can take place between Ps and the molecules involved, *i.e.* π-π, dipole-dipole, hydrogen bonding and ionic interactions. Since they present aromatic characteristics, the reversed-phase type seems to be the predominant interactions with the neutral molecules at pH 5.5. From the data achieved, the AC2 sorbent was chosen for further assays once exhibit the best compromise, with simultaneously acceptable selectivity demonstrated (> 50%) for the target analytes, when compared with the remaining sorbent phases tested, under similar experimental conditions.

3.2.3. Improvements on the back-extraction performance

For the present work, it was our goal to improve the back-extraction step by reducing the volume of the desorption solvent in several orders of magnitude, *i.e.* at the microliter level and, simultaneously, the elimination of the solvent switch step. These improvements turn possible saving time, making easier the practical manipulation and more environmentally friendly. Therefore, a micro-liquid desorption (µLD) step was introduced by using a microliter insert full (200 µL) with a suitable solvent. This novel approach must use a solvent with enough strength for the complete stripping of the target compounds from the sorbent phase, as well as, compatibility with the instrumental sample injection system [29]. Fig. 2(b1) shows images where this new improvement can be observed, in which the analytical device is totally immersed in a suitable solvent inside the insert. Thus, solvents such as ACN, MeOH, mixtures of ACN/MeOH (1:1, v/v) and *n*-C6 were tested, covering a wide range of polarity that allow the best µLD performance, which guarantee the complete removal of the insecticide repellents after BAµE(AC2). Fig. 4(a) depicts the back-extraction profile of the solvents tested under sonification, where is clearly seen that the best recoveries are attained with ACN for the insecticide repellents under study. This approach proves that if the solvent selected has enough capacity and convenient volume, the solvent switch step can be discarded as previously proposed. Moreover and since the µLD time can be also a critical back-extraction parameter, several assays were performed having different periods of time (15–60 min), where negligible variation was observed (data not shown). As a consequence, 15 min was selected for further studies. The carryover effect was also evaluated through series of µLD replicates, where the background was always below the instrumental LODs achieved. From the data obtained we can anticipated that this novel improvement on the back-extraction stage in a single step is effective, simple, and easier to work-up and in accordance with the green chemistry principles. Nevertheless, it is peremptory the selection of the right µLD solvent, taking into consideration the strength capacity for desorption purposes, as well as, the compatibility with the instrumental sample injection system involved.

3.2.4. Optimization of the BAµE(AC2) performance

It is well known, that agitation speed can cause a significant effect on the extraction efficiency, because influence the diffusion of the mass transfer process of the insecticides towards the sorbent phase, thus affecting the microextraction performance, in particular during the floating sample approach [16,17,26]. As it has been showed in previous works [19–21,23,30–32], high stirring rates are usually avoided, because decrease the efficiency yields, depending on the magnet size. For stirring rates above 1000 rpm, the magnetic stir bar becomes unstable creating, therefore, higher turbulence and affecting the rotational motion of the BAµE analytical device, leading to bad precision. The profile attained for assays performed at 750, 1000 and 1250 rpm (data not shown) indicates an advantage for 1000 rpm, which was chosen for further experiments. During the BAµE process, the interaction between the analytes in the sample bulk and the sorbent phase is based on an equilibrium process. Such phenomenon is affected by some kinetic parameters, whereas the agitation speed but also the equilibrium time is very important [18–23] since can affect the efficiency yields. Thus, the equilibrium time

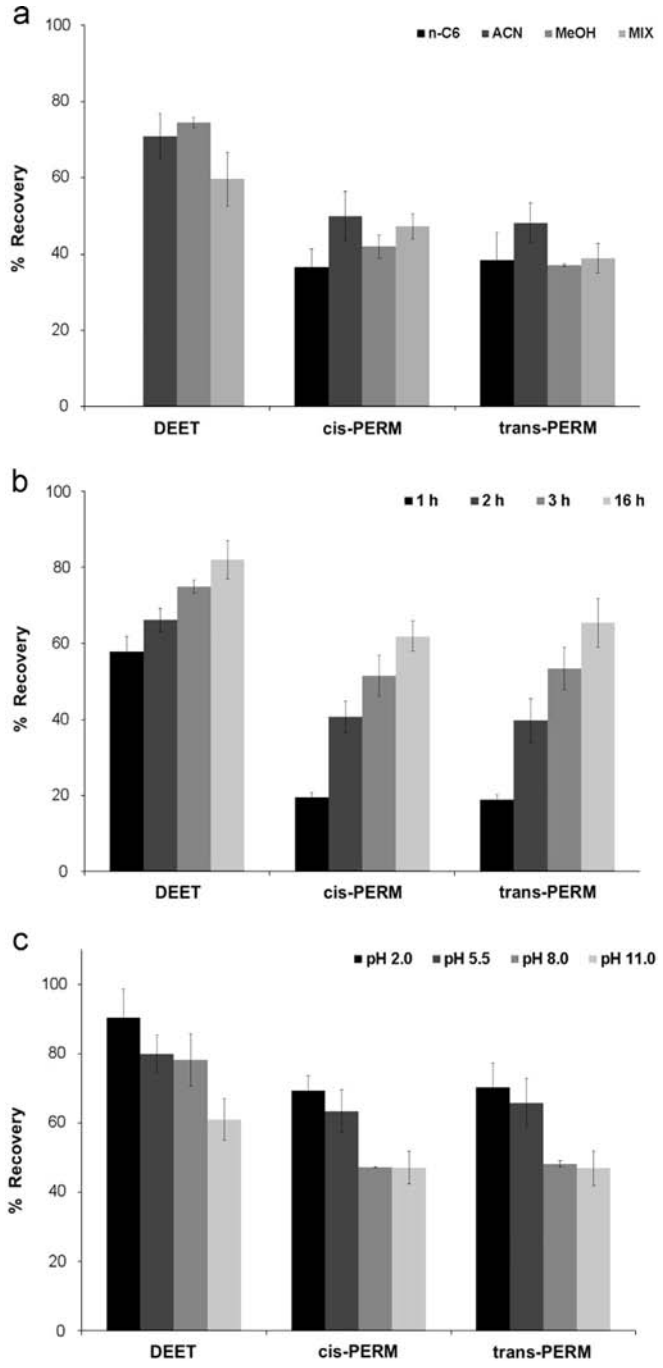

Fig. 4. Effect of the solvent type (a) on the back-extraction, equilibrium time (b) and pH (c) on the extraction efficiency of the insecticides by BAμE(AC2)-μLD/LVI-GC–MS(SIM). (BAμE(AC2): 25 mL water sample (1.0 μg/L), 3 h (16 h for the pH study), 1000 rpm, pH 5.5; μLD: ACN (200 μL), 15′ under ultrasonic treatment).

exposure of analytes to the microextraction device sorbent is probably one of the most important parameter that may limit the compounds distribution between the two phases during the process, having a strong effect on the recovery yields. Assays involving equilibrium times in between 1 and 16 h were performed for the target insecticides at room temperature. Fig. 4(b) shows the profile obtained, where it is notice that the equilibrium kinetics present a slow behaviour for the three target analytes, once 16 h allows obtaining much better efficiency yields. Therefore, the period of time of 16 h was selected for further studies. Although higher equilibrium times are demanded (16 h) to reach the steady state conditions, another advantage comparatively to other enrichment techniques, is that BAμE allows the possibility to operate overnight, without any special requirements. Subsequently, the characteristic of the sample matrix was assessed, in particular the pH, ionic strength and polarity. Preliminary assays consisted in the pH effect on the BAμE(AC2) efficiency for the insecticides under study, where several values (2.0, 5.5, 8.0 and 11.0) were assessed at room temperature. As stated before, this parameter has already been described to have a strong influence, since the recovery yields are affected by the ionic or neutral form of the target compounds in the sample bulk, as well as, the surface charge of the AC2 phases [19,20]. For DEET in particular, the neutral form occurs, predominantly, at pH values higher than 2.0, and below this value, the positively charged form takes place since the nitrogen atom become protonated. On the other hand, *cis* and *trans* PERM are very stable species and do not suffers ionization. Fig. 4(c) depicts the data obtained from the pH effect evaluation, where it is clearly observed that the optimum value is attained at 2.0, where much better recoveries are reached. At pH 2.0 the net charge of AC2 is almost neutral ($pH_{Sample} \approx pH_{PZC}$), which is favourable for both PERM isomers and DEET. Nevertheless, since at pH 2.0 the DEET has around 8% of the positive ionic species formed, the recovery of around 90% can be due to electrostatic and/or dispersive interactions. For PERM (*cis*- and *trans*-), although both species do not ionize, for higher pH values the recoveries decreased because AC2 net surface become negative, which is less favourable for all the three insecticide repellents having neutral forms. Therefore, the best compromise to force the migration of insecticides to the sorbent phase was to established pH 2.0 to proceed for the study of the next parameters. The addition of an electrolyte increases the ionic strength and favours the migration of organic compounds toward the sorbent phase, which can strongly affect the efficiency yields, especially for the more polar ones ($\log K_{O/W} < 3$). In general, the addition of salt increases the recoveries of the more polar targets, because the "salting-out effect" is based on decreasing the solubility of the compounds forcing them to migrate to the sorbent [19,20,30]. Additionally, the polarity of aqueous matrix can also be controlled by the addition of an organic solvent that can reduce the "wall-effect". The analyte adsorption on the vial glass walls is also a phenomenon that could promote decreases in sorption efficiency, particularly for the most hydrophobic compounds at trace levels [33]. Therefore, assays were performed with the addition of NaCl (up to 15%, w/v) and MeOH (up to 15%, v/v) in the aqueous media. From the results obtained (data not shown), a slight increment of the average efficiency occurs for DEET at 10 and 15% of NaCl, but decreases significantly the recovery of PERM isomers. Regarding the MeOH additions, the opposite is observed, where the efficiency of DEET is strongly reduced and for PERM forms (cis- and trans-) is negligible. In short, we can conclude that the polarity at this stage plays a very important role on the efficiency assays performed. Thus, once DEET present hydrophilic characteristics, the recovery yields is influenced through the ionic strength by forcing the molecules towards the sorbent phase, whereas the PERM species are influenced by the "oil-effect" since they are hydrophobic. On the other hand, when MeOH is added the matrix become more organic, decreasing the DEET recovery since it increases the solubility, although negligible effect is observed for PERM (*cis*- and *trans*-) species. As a consequence, the further assays were performed in absence of both salt and organic modifier.

3.3. Validation of the BAμE(AC2)-μLD/LVI-GC–MS(SIM) methodology

After the optimization of the best experimental conditions for the repellent insecticides under study, the steady state conditions can be established as: BAμE(AC2): 16 h (1000 rpm), pH 2.0; μLD: ACN (200 μL), 15 min with sonification. Subsequently, assays

performed on 25 mL of ultrapure water samples spiked with the target insecticides at the 1.0 μg/L level, showed that the proposed methodology presents good performance, with average recovery yields of 96.4 ± 9.9% for DEET, 76.5 ± 13.9% for *cis*-PERM and 73.8 ± 8.8% for *trans*-PERM, under optimised experimental conditions. The linear dynamic ranges for the present methodology was also assayed on ultrapure water samples, where the target analytes having concentrations between 0.04 and 4.0 μg/L presented good linearity ($r^2 > 0.9963$; DEET) and suitable precision (RSD < 15.2%; 1.6 μg/L, *trans*-PERM). Furthermore, the sensitivity of the methodology was also checked, where LODs of 8 ng/L (PERM *cis* and *trans*) and 20 ng/L (DEET), and LOQs of 26 ng/L (PERM *cis* and *trans*) and 66 ng/L (DEET) were achieved, calculated at an S/N of 3 and 10, respectively. Table 2 summarizes the experimental average recoveries, the linear dynamic ranges, determination coefficients, LODs and LOQs for the target compounds through BAμE(AC2)-μLD/LVI-GC–MS(SIM), under optimised experimental conditions. Intraday and interday repeatability assays were also evaluated for the present methodology, calculated as RSD on 3 and 9 assays (in three different days), respectively. For intraday repeatability, good agreement in efficiency ranged from 64.3% (*cis*-PERM, 1.6 μg/L) to 98.4% (DEET, 1.6 μg/L) were achieved for all concentrations used, as well as, good precisions (RSD) were attained from 6.3% (*trans*-PERM, 0.6 μg/L) to 14.9% (*trans*-PERM, 1.6 μg/L). For interday repeatability assays performed at 1.0 μg/L level, the efficiency achieved was 96.7 ± 9.0% for DEET, 72.7 ± 10.5% for *cis*-PERM and 70.2 ± 11.9% for *trans*-PERM. Fig. 5 exemplifies a total ion chromatogram relative to an assay performed on ultrapure water spiked at the 1.0 μg/L level, obtained by BAμE (AC2)-μLD/LVI-GC–MS(SIM), under optimised experimental conditions. In short, the novel improvements on the methodology developed herein, demonstrated excellent robustness and reproducibility due to the good analytical data achieved. Although more environmentally friendly, it is much easier to work-up and efficient for trace level analysis of insects repellent, allowing a remarkable selectivity and sensitivity for matrices with great complexity such as environmental water.

Table 2
Average recoveries, LODs, LOQs, linear dynamic range, and determination coefficients achieved for three insecticides obtained by BAμE(AC2)-μLD/LVI-GC–MS(SIM), under optimised experimental conditions.

Insecticides	Recovery[a] (% ± RSD)	LODs[b] (ng/L)	LOQs[c] (ng/L)	Linear range (μg/L)	r^2
DEET	96.4 ± 9.9	20	66	0.08–4.0	0.9963
cis-PERM	76.5 ± 13.9	8	26	0.04–4.0	0.9965
trans-PERM	73.8 ± 8.8	8	26	0.04–4.0	0.9972

[a] Assays performed at the 1.0 μg/L level; $n = 3$.
[b] LODs at S/N = 3.
[c] LOQs at S/N = 10.

3.4. Improvements on downsizing the analytical device

Although good analytical data was obtained through the optimised methodology proposed, it was also our strategy downsized the BAμE device in order to test and compare the analytical performance, under optimised experimental conditions. Thus, we reduced the device to half-size (½BAμE(AC2); Fig. 2(a2)) to verify the analytical efficiency of

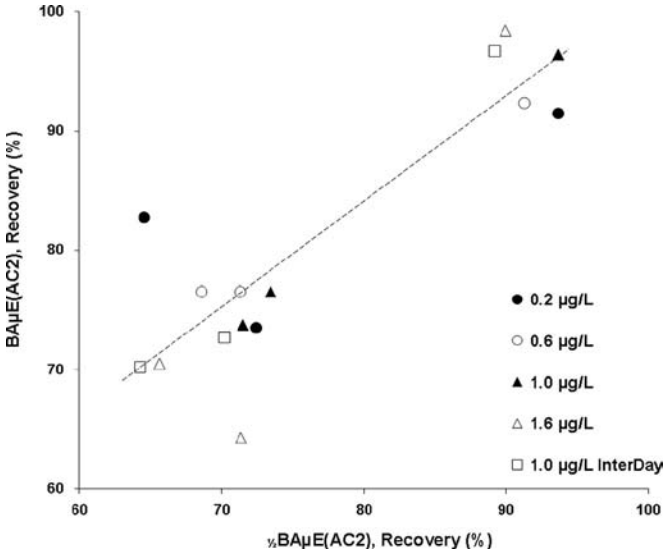

Fig. 6. Comparison between BAμE(AC2) against ½BAμE(AC2) methodologies on the efficiency yields for the three insecticide repellents followed by μLD/LVI-GC–MS (SIM), under optimised experimental conditions.

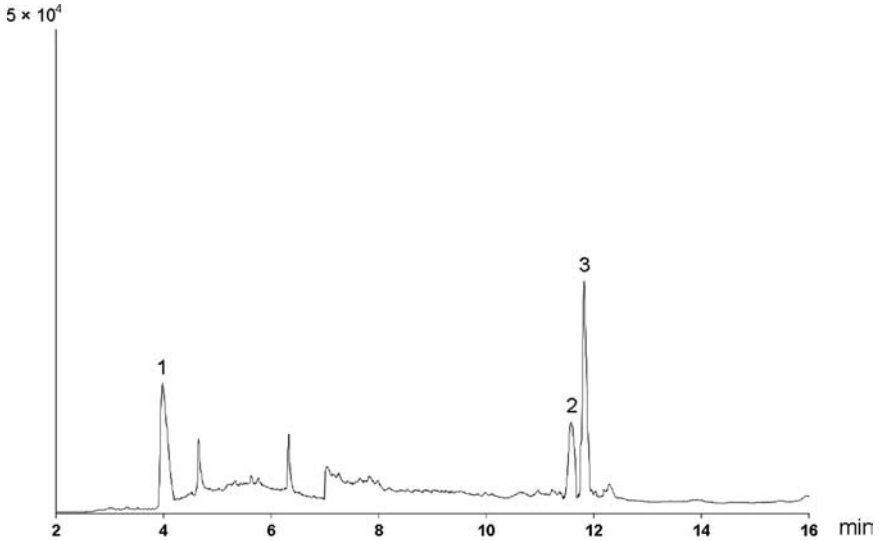

Fig. 5. Total ion chromatogram exemplifying an assay performed on spiked ultrapure water (1.0 μg/L) by BAμE(AC2)-μLD/LVI-GC–MS(SIM), under optimised experimental conditions. (1: DEET, 2: *cis*-PERM and 3: *trans*-PERM).

Table 3
Slopes and determination coefficients achieved using the SAM in tap, ground, estuary, river and swimming-pool water samples, for the three insecticides obtained by BAμE (AC2)-μLD/LVI-GC–MS(SIM), under optimised experimental conditions.

Insecticides	Tap		Ground		Estuary		River		Swimming-pool	
	Slope	r^2	Slope	r^2	Slope	r^2	Slope	r^2	Slope	r^2
DEET	361,661	0.9955	307,158	0.9943	225,306	0.9951	817,515	0.9967	251,840	0.9962
cis-PERM	239,352	0.9953	266,112	0.9973	184,375	0.9943	426,093	0.9960	158,083	0.9946
trans-PERM	1×10^6	0.9958	1×10^6	0.9979	815,407	0.9955	2×10^6	0.9941	619,741	0.9944

half amount of the sorbent phase during the extraction stage, as well as, the increment of the phase ratio with the desorption solvent during the back-extraction stage (Fig. 2(b2)). Therefore, assays were performed at different concentration levels in the same and three different days, to demonstrate if the analytical efficiency is or not affected. Fig. 6 depicts the comparison from intraday and interday repeatability assays performed by BAμE(AC2) against ½BAμE(AC2) devices, under similar experimental conditions. The data obtained from the intraday repeatability assays shows that the ½BAμE(AC2) recoveries ranges from 64.5 ± 9.5% (*trans*-PERM, 0.2 μg/L) to 93.7 ± 6.3% (DEET, 0.2 μg/L), while BAμE(AC2) are in between 64.3 ± 13.5% (*cis*-PERM, 1.6 μg/L) and 98.4 ± 7.9% (DEET, 1.6 μg/L). From the interday repeatability assays performed at 1.0 μg/L, the ½BAμE(AC2) recoveries were 89.2 ± 9.5% for DEET, 70.2 ± 12.4% for *cis*-PERM and 64.3 ± 11.3% for *trans*-PERM, while by BAμE(AC2) were 96.7 ± 9.0% for DEET, 72.7 ± 10.5% for *cis*-PERM and 70.2 ± 11.9% for *trans*-PERM. By comparing the analytical efficiency obtained through these two analytical devices, we can conclude that both are equivalent since negligible differences are notice. For ultra-trace analysis or in case of small sample volume (*e.g.* < 5 mL), we can also anticipate that the downsizing of the BAμE(AC2) device could be a suitable alternative, which is compatible with the reduction of desorption solvent at the microliter level. The data also demonstrate that the large specific areas exhibited (up to 1000 m^2/g) by these materials present a remarkable adsorptive capacity (≈ 100–500 μg/mg), which are definitely below the isothermal plateau (saturation of the sorbent) and therefore, the Langmuir and Freundlich theoretical considerations are not applicable [26]. Finally, and in the experimental point of view, the smaller is the device the higher phase ratio will be attained with the desorption solvent, which turns the back-extraction stage much more effective for many cases.

3.5. Application to real matrices

To demonstrate the practical ability of the present methodology to real samples, several assays were applied to environmental matrices such as tap, ground, river, estuary and swimming-pool waters. Due to the complexity nature of real samples, the SAM method was used for quantification purposes, as well as, to compensate for possible matrix effects, according to previous reports [18–23]. Assays were performed by spiking the real samples with four working standards having concentrations ranging from 0.6 to 2.8 μg/L. Blank assays (C_0) were also performed without spiking to guaranty minimum matrix interference and maximum control of the analytical methodology. The results obtained are summarized in Table 3, where good linearity ($r^2 > 0.9941$, *trans*-PERM) was obtained for all samples studied. From the data obtained, the slopes of tap and ground waters present the same order of magnitude, where the highest sensitivity was achieved for the river water matrix. Nevertheless, the sensitivity was significantly reduced for the estuary and swimming pool water samples, which can be attributed to substantial matrix effects presented through the salt content. Although the proposed methodology showed very high sensitivity at the ultra-trace level, these insecticide repellents were not detected (< LODs) in the real samples studied.

4. Conclusions

The methodology proposed (BAμE(AC2)-μLD/LVI-GC–MS(SIM)) in the present study, using a very selective activated carbon phase, was fully optimised and validated to monitor simultaneously three insecticide repellents (DEET, *cis* and *trans* PERM) in environmental water matrices. Under optimised experimental conditions, a remarkable analytical performance was attained, including accuracy, precision, suitable detection limits and excellent linear dynamic ranges. All the novel improvements proposed, particularly the back-extraction stage performed in one single step with reduced solvent volume, as well as, the downsizing of the BAμE device, demonstrate excellent performance, turning possible to save time, making easier the practical manipulation and more environmentally friendly. The application of the present methodology to monitor traces of these insecticide repellents in tap, ground, river, swimming-pool and estuary water samples provided very good performance through the SAM. The method presents robustness is easy to implement, require low sample volume, presenting a very high selectivity and sensitivity to monitor trace levels of insecticide repellents in environmental water matrices. This new analytical approach that uses nanostructured particles and operates under the floating sampling technology, has proved to be a suitable alternative, in which allows to tuning the coating phase selectivity according the target analytes under study, whenever other sorption-based static microextraction techniques present lack of effectiveness.

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