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Green determination of brominated flame retardants and organochloride pollutants in fish oils by vortex assisted liquid-liquid microextraction and gas chromatography-tandem mass spectrometry

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ABSTRACT

. A "green", simple, and low-cost sample extraction procedure involving the use of a deep eutectic solvent (DES) in a vortex assisted liquid-liquid microextraction (VALLME) technique followed by gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis was developed for the simultaneous determination of different PBDEs congeners and OCPs residues in fish oils. After evaluation of different eutectic mixtures, the extraction parameters (volume of DES, amount of oil sample and extraction time) were optimized by means of experimental design in order to maximise extraction efficiency. The developed method was validated in terms of linearity, accuracy and precision, presenting limits of detection in the low ng g^{-1} level. Its application in the analysis of five fish oil samples, allowed the detection of all the target analytes at levels up 21.5 ng g^{-1}. Fish oils used in animal feed showed to be more contaminated than fish oils for human consumption.

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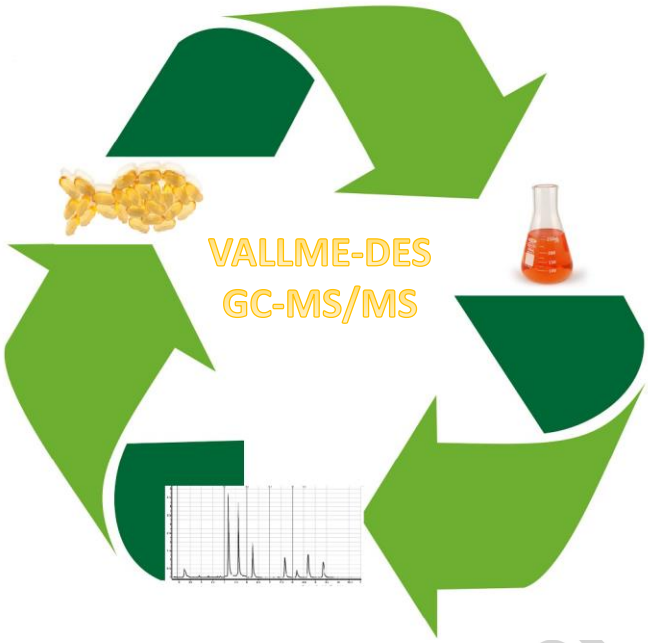
Graphical Abstract:



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KEYWORDS

Choline chloride:phenol; Deep eutectic solvents; GC-MS/MS, PBDEs, OCPs, Food contaminants

INTRODUCTION

Over the past 70 years, persistent organic pollutants (POPs) including organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), polychloro biphenyls (PCBs), and polychlorinated dibenzodioxins and dibenzofurans (PCDDs/PCDFs) have been released into the environment through anthropogenic activities, being nowadays dispersed all over the world, in the different environmental compartments. Overall POPs are characterized by low water solubility and high lipophicity, which gives them high potential for bioaccumulation in fatty tissues of living organisms, reaching all trophic levels [1,2]. Moreover, these compounds have a significant negative impact on human and animal health, causing different adverse effects such as endocrine disruption [3], reproductive [4] and neuronal disturbances [5], and carcinogenesis [6].

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OCPs, such as hexachlorobenzene (HCB) or dichloro-diphenyl-trichloroethane (DDT), were for a long time widely used in agriculture due to their efficacy against harmful insects. Currently, their application is banned or strongly restricted, owing to their persistence in the environment and bioaccumulation potential [7,8]. PBDEs are synthetic chemicals that have been widely used in many industrial products to reduce its flammability and thereby prevent or retard fire spread. Because PBDEs are mixed into polymers and not chemically bound to other components, they may separate from the products, leaching into the environment [9]. The Stockholm Convention have decided to list the most dangerous PBDEs (Deca-BDEs, Penta-BDEs and Octa-BDEs), as well as HCB and DDT as POPs substances, in order to the parties take measures to eliminate both the production and use of these toxic chemicals [10].

Human exposure to OCPs and PBDEs occur mainly through the consumption of contaminated food, especially fatty fish. Since some fish varieties could have a relatively high lipid content, elevated concentrations of POPs can be present in those fish and derivative products including fish oils. Currently, extraction of PBDEs from fish oils supplements are commonly performed by accelerated solvent extractor [11] and solid liquid extraction (SLE) [1-1] followed by gel chromatography column [13,14] or sulphur treatment [12, 15] as cleanup. In case of OCPs, SLE and Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) are the most common extractive techniques [15-17]. Despite QuEChERS provided a huge reduction in time and solvent consumption compared with SLE, this approach provides no additional enrichment factor making it difficult to achieve stricter limits of quantification [18]. The current trend in contaminant analysis is to employ microextraction approaches, with low solvent consumption and high enrichment factor such as dispersive liquid-liquid microextraction (DLLME) [19] and other similar techniques such as air assisted liquid-liquid microextraction (AALLME) or vortex assisted liquid-liquid microextraction (VALLME). In all these techniques a small amount of extractant (water immiscible solvent, added or not with a dispersive solvent) is rapidly injected into the aqueous sample to form a cloudy solution promoting the easy interaction of fine droplets of extractant with the analytes. To the best of our knowledge the simultaneous extraction of OCPs and PBDES with an LLME approach has not been so far applied in fish oil analysis.

The solvents currently employed in all extraction techniques above mentioned are classified as volatile organic compounds and possess a negative impact on health, safety and environment [20]. To surpass these drawbacks, scientific effort has been made to develop ecological friendly and sustainable solvents. Deep eutectic solvents

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(DESs) firstly proposed by Abbot [21] match well with what is intended. DESs are eutectic mixtures prepared by simply mixing two or more inexpensive naturally occurring compounds, which are non-toxic and totally biodegradable. The availability, low cost, biodegradability and environmental friendliness of the components makes the DESs versatile alternatives to conventional organic solvents [22, 23].

DESs have been applied for extraction and separation of bioactive phenolic compounds, saponins and flavonoids from plant materials and vegetable oils [23-26]. Recently, Farajzadeh et al. used a DES mixture (choline chloride/4-chlorophenol) as extractant for extraction of pesticide residues from fruit and vegetable juices [27]. Nevertheless, to the best of our knowledge there are no reports on their use for extraction of POPs from fish oil supplements.

The aim of this work was to evaluate the performance of hydrophilic DESs as an alternatives extractant in a VALLME procedure designed for the simultaneous extraction of several prevalent PBDEs (28, 47, 99, 153 and 154) and OCPs (HCH, HCB, 4,4 DDT) in fish oils followed by gas-chromatography tandem mass spectrometry (GC-MS/MS) analysis. Several hydrophilic DESs solvents were evaluated and an experimental design was conducted to optimize different VALLME parameters. The results from the design were evaluated with the analysis of variance to determine the statistical significance of the main factors of extraction and interaction effects of these factors. The method developed was validated and applied to different fish oils supplements as a demonstration of its applicability in monitoring studies.

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EXPERIMENTAL SECTION

Reagents and solutions

Individual standards of PBDE (each at 50.0 µg mL^{-1} in nonane, purity ≥98%) and OCPs ("pestanal" purity) were obtained from Wellington laboratories (Canada) and Sigma-Aldrich (Steinheim, Germany), respectively. Working standard solutions with mixture of: 2,4,4′-TriBDE (BDE-28), 2,2′,4,4′-TetraBDE (BDE-47), 2,2′,4,4′,5-PentaBDE (BDE-99), 2,2′,4,4′,5,6′-HexaBDE (BDE-154), 2,2′,4,4′,5,5′-HexaBDE (BDE-153), hexachlorobenzene (HCB), α-hexachlorocyclohexane (α-HCH) and 1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (4,4-DDT) were prepared in toluene at 5 µg mL^{-1} Internal standards (ISs) 5′-fluoro-3,3′,4,4′,5-pentabromodiphenyl ether (FBDE-126, 50 µg mL^{-1} in isooctane) and triphenylphosphate (TPP, purity ≥98%) were purchased from

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AccuStandard (New Haven, CT, USA) and Sigma-Aldrich, respectively. Working ISs of FBDE-126, to final concentration of 100 ng mL^{-1} and TPP to final concentration of 250 ng mL^{-1} were prepared in toluene. All the solutions were stored at 4ºC when not in use.

Choline chloride (ChCl, purity ≥98%), phenol (Ph, purity≥ 99%), glycerol (Gly, purity ≥99.5%), urea (Ur, purity for synthesis), DL-lactic acid (Lac, purity ≥90%) and ethylene glycol anhydrous (Eg, purity ≥99.8%) used in DES preparation were all purchased from Sigma Aldrich.

Ethanol, methanol (MeOH) and toluene were of HPLC grade and were purchased from Merck (Darmstadt, Germany).

Sampling

Five samples of different fish oils were analyzed. One refined sardine oil (sardine_AF) was provided by Industrias Afines S.L. (Spain) and one mix refined tune and sardine oil (tuna_AF) was provided by AFAMSA S.L. (Spain). Both are considered as animal feed quality. Two omega-3 concentrates (n3-HF1 and n3-HF2, from tuna and salmon, respectively) currently commercialized in capsules for human supplementation were obtained in local markets of Burgos (Spain) and one omega-3 concentrate capsule (n3-HF3) was obtained in a local market of Porto (Portugal).

Preparation of DES

All DESs were prepared according to the methodologies described in literature [22,23]. Briefly ChCl (2 g), as hydrogen bond acceptor (HBA), was mixed with different compounds as hydrogen bond donors (HBD) at a proper molar ratio in a 40 mL screw-cap tube with a mechanical stirrer at 350 rpm and 60°C, until a clear and homogeneous liquid was formed. The compounds of DES mixture were weighed in an analytical high precision balance with an resolution of ± 10^{-4} under a controlled atmosphere box. **Table 1** listed the abbreviations of the DESs produced in this work.

GC-MS/MS conditions

The chromatographic analysis was performed using an Agilent 7890B gas chromatograph, equipped with an Agilent 7693A autosampler with multimode inlet (MMI), coupled to a triple quadrupole mass spectrometer, 7000C (Agilent Technologies Inc., Palo Alto, CA, USA), with an EI source working at 320°C in electron capture positive ionization mode. The GC separation was performed using a ZB-5MS capillary

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column (30 m × 0.32 mm ID and a film thickness of 0.25 µm, Phenomenex, Torrance, CA, USA) working at a constant flow of 1.3 mL min^{-1} of helium (99.999%; Gasin, Portugal). The oven program was set as follows: 150 °C (1.5 min); 40 °C min^{-1} to 250 °C; then 7 °C min^{-1} to 320 °C, stay 1.5 min with a total run time of 15.5 min. **Figure S1** shows a Total Ion Chromatogram of a standard solution with all the analytes in study and ISs. The injection of 1 µL of sample extracts was performed in pulsed splitless mode (purge-off time 60 s) at a temperature of 300°C.

The MSD was operated using electron ionization (70 eV) in the multiple reaction monitoring (MRM) mode. The MRM conditions and retention times (tR) for the selected analytes are presented in **Table S1**. Dwell times were set between 10 to 22 ms. The MSD transfer line was at 250 °C, ion source at 320 °C, and quadrupoles at 150 °C. Helium was used as the quench gas (at 2.25 mL min^{-1}) and nitrogen as the collision gas (at 1.5 mL min^{-1}). Agilent MassHunter software was used for instrument control and data analysis.

Sample preparation

An aliquot of sample (300 mg) spiked with 24 µL of TPP at 250 µg L^{-1} was mixed with 180 µL of DES: EtOH (1:1 v/v) solution. Then, the mixture was vortexed for 5 min until a cloud solution was formed. After centrifuged at 2000 *g* for 3 min, 50 µL of the lower phase was transferred to an insert tube and mixed with 10 µL of FPBDE (IS) 126 at 250 µg L^{-1}.

Quality assurance/quality control

Taking into account the widespread use of PBDEs at relevant concentrations in daily life products, laboratory contamination can be a common situation. Therefore, plastic material was avoided to prevent contamination. Blank samples were analyzed for each batch of samples to verify the background contamination.

Extra-virgin olive oil (free of the analytes of interest - blank sample) obtained from a local market on Porto (Portugal) was used as food matrix to carry out the experiment design, to optimize the extraction conditions. Calibration curve (CC) standards and quality control (QC) samples were prepared by spiking virgin olive oil (blank sample) with working solutions. CC were prepared at 1.0, 2.5, 5.0, 12.5, 25.0, 50.0, 75.0 and 100.0 ng g^{-1} while QC were prepared at three different levels: low: 5.0 ng g^{-1} (LQC), medium: 25.0 ng g^{-1} (MQC); and high: 75 ng g^{-1} (HQC) concentrations.

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Statistical analysis

Experimental design was performed using Statgraphics Centurion XVII software (StatPoint Technologies, Inc. USA). After determining the range of extraction variables on the basis of preliminary single-factor test, a multilevel factorial design was used to find the optimal values for three independent variables: amount of DES (designed as A), amount of oil sample (designed as B), and extraction time (designed as C). Whole complete design included 21 experiments, 18 different combinations of the variables and 3 more as replications of some of them. The experiments were performed in a random manner at different combinations of these parameters using statistically designed experiments. The extraction efficiency (recovery %) was taken as the response of the design experiments. Regression analysis was carried out according to the experimental data. A 95% confidence level was used for the statistical analysis of the data.

RESULTS AND DISCUSSION

Optimization of the Extraction Conditions

Initially, several DESs were prepared by mixing ChCl with different HBD at a proper molar ratio, and the respective performance as VALLME extractors was evaluated in duplicate under the same conditions: 500 mg of extra-virgin olive oil samples (blank sample) spiked with PBDEs and OCPs at 100 ng mL^{-1} each was extracted with 100 µL of DESs during 5 min, using the action of vortex to promote the cloud formation. **Figure 1** shows the chromatograms obtained from nine different DESs mixtures that were assayed. A significant difference in the extractability of both PBDEs and OCPs with the tested DESs were reflected in the analytical signal obtained for each analyte in the GC-MS/MS. The best results were obtained with ChCl-Ph-2 followed by ChCl-Gly and ChCl-Lac-1. In general, all the mixtures tested can be considered hydrophilic, although they presented great differences in terms of viscosity, density and polarity, among other features. In LLME techniques is convenient to select extractant with low viscosity to facilitate droplets formation and consequently to enhance the surface of contact between the extractant and the matrix, along with a density quite different from the density of the matrix, to allow a good separation between the two phases at the final step. For example, the tested mixture of ChCl-Ur is very dense and viscous (1.25 g cm^{-3} and 449 mPa·s at 303.2 K[28]) when compared to the other synthesized DESs (**Table S2**).In fact, ChCl-Ur was not able to extract most of analytes as it can be observed in

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the chromatogram E) of **Figure 1**. Additionally, the high polarity of some mixtures, such as ChCl-Eg, ChCl-Ph-3 or ChCl-Ph-4, can explain the poor extraction yields obtained when compared with ChCl-Ph-2 [24]. Therefore, further experiments were performed with ChCl-Ph-2 due to its best analytical signal for all the analytes.

Despite the good extraction efficiency exhibited by ChCl-Ph-2 as extractant some issues came across GC-MS/MS injection. The extract obtained was unable to provide the reproducibility of the autosampler injection of 1 µL due to its high viscosity. To overcome this drawback two different solvents namely methanol and ethanol were mixed with ChCl-Ph-2 in an equal proportion (1:1, v/v), previous to the VALLME. As presented in **Figure 2** as lightly higher extraction efficiency was observed with ChCl-Ph-2/ethanol, additionally with a good intermediate precision relative standard deviation (RDS%, N=5) lower than 15% for all the analytes. Thus, based on the above described results, the mixture of ChCl-Ph-2 (choline chloride:phenol; 1:2 molar ratio) plus ethanol at 1:1 (v/v) was applied in further experiments.

Effect of DES volume, sample amount and extraction time. Experimental design

The extraction efficiency in VALLME is affected by other parameters besides the type of extraction solvent, such as volume of extractor, volume of sample and extraction time. To evaluate and optimize some VALLME conditions a statistics-based design was performed, with overall scope from fewer experiments. The factors and levels selected were the following: volume of DES (A) at three levels (80, 130 and 180 µL); amount of oil sample (B) at three levels (300, 500 and 700 mg); and extraction time (C) at two levels (10 and 30 min). In order to evaluate the efficiency of the proposed procedure, the extraction recovery of POPs was considered as the response. The experiments were performed in random order to avoid systematic error. The design matrix including all the experiments and the related responses is shown in **Table S3**.

The model quality was evaluated in terms of the square of the correlation coefficient (R^2) and the lack-of-fit was evaluated by analysis of variance (ANOVA) at the 95% confidence level. The resulting R^2 value were 0.8351, indicating that the experimental data were in relatively good agreement with predicted extraction yields for the model. As it can be seen in **Table 2**, A, B and the interaction AB resulted to be statistically significant at the 95% confidence level, having *P*-values < 0.05. The significance of interaction between A and B means that the amount of extractant and sample are

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dependent on each other. On other hand time (C) in the range considered was not an important factor.

For graphical interpretation of the significant interactions between the variables, a contour of estimated response surface of the model was considered. **Figure 3** presents the contour plot showing the effect of DES volume (µL) and amount of oil (mg) on extraction efficiency (%), without considering the extraction time, the third effect evaluated, and its interactions, AC and BC, as not statistically significant factors. As it can be observed, when the DES volume increases with lesser amount of oil, the extraction efficiency increases. Recoveries reached a maximum when 300 mg of oil and 180 µL are used.

Method validation

To evaluate the analytical performance of the developed method, a series of experiments were designed to assess matrix effect, linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ), following the guidelines Sante 2017 [29].

The matrix effect is caused by the presence of interferents in the sample that could lead to a suppression or enhancement of the analytical signal. Taking into consideration the chemical diversity of analytes that we intended to analyzed and the high lipid content of the samples, matrix effect was tested before all the other parameters. By comparing calibration curves in matrix and solution (toluene) a significant matrix effect was observed, all the analytes presenting lower slope in matrix than in solution. Therefore, linearity response was performed by analyzing matrix-matched standards (olive oil sample free of POPs in study) at eight concentration levels in the range of 1−100 ng g^{-1}, and employing TPP as surrogate standard and FBDE-126 as internal standard. For all the analytes, the obtained calibration functions were linear with r values in the range of 0.9903−0.9979 (**Table 3**).

Precision was expressed as percentage of the relative standard deviation (%RSD) of repeatability and intermediate precision (**Table 3**). Both parameters were determined by analysing in sextuplicate samples spiked at three concentration levels (5, 25 and 75 ng g^{-1}) in the same day (repeatability) or in five days (intermediate precision), according with European Commission (EC) guidelines [30]). The RSD repeatability were <16% and the intermediate precision repeatability was <21%, which are satisfactory since RSD never exceeded 20% with the exception of 4,4-DDT.

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For the evaluation of the efficiency of the extraction and the accuracy of the analytical procedure developed, 300 mg of Sardine_AF and Tuna_AF were spiked at three different levels - 5, 25 and 75 ng g^{-1} - of a standard mixture (PBDEs and OCPs) and analyzed in triplicate. The relative recoveries calculated as the ratio between added and found analyte concentrations are presented in **Table S4.** Average recovery values at each fortification level were within the range of 76 ±11, 84 ±12 and 90 ±9 for 5, 25 and 75 ng mL^{-1}, the measured values are within the control limits established by SANTE (recoveries should be between 70% and 120%, with RSD ≤ 20%) [29]. Calculation of a reasonable estimate of the total uncertainty for a measurement result obtained with the method under study fish oil samples was accomplished considering the data from measurement performance characteristics. The target standard combined uncertainty ($u'_c{}^{tg}$), reflecting the combination of precision ($u'_{ra}{}^{tg}$) and uncertainty on bias ($u'_{sy}{}^{tg}$) was calculated with the showed formula (1), where $u_{ra}{}^{tg}$ was estimated from the defined target value for the coefficient of variation (CV), and $u_{sy}{}^{tg}$ was estimated from the maximun/minimum permissible mean error (of R max = 120% and R min = 70%) [30]:

$$u'_c = \sqrt{(u'^{tg}_{ra})^2 + (u'_{sy}{}^{tg})^2} \quad (1)$$

$$u'_{sy}{}^{tg} = (Rmax - Rmin)/2*6^{1/2}$$

The target standard bias uncertainty component ($u_{sy}{}^{tg}$) on measurements not corrected for mean error was calculated by reducing the confidence level of the half error range by a factor of $6^{1/2}$ (triangular distribution for the mean error range with 100% confidence level). As can be observed in Table S4 a measurement uncertainty value under than 18.4% was obtained, which is lower than the target value of 25% setting from the evaluation criteria European Union proficiency tests.

The limit of quantification (LOQ) was determined based on the criteria given by the guidelines Sante 2017 [29] as the minimum concentration that can be quantified with acceptable accuracy and precision, which was the lowest calibration level of the calibration curve. The limit of detection (LOD) of the method was determined from the analyses of a zero sample spiked at 1 µg kg^{-1} for each analyte. The LODs for the POPs based on a signal-to-noise (S/N) ratio of 3 ranged from 0.2 to 0.7 ng g^{-1} (**Table 3**). The results of LODs obtained by the proposed procedure were comparable with those of several previously reported SPE procedures for the determination of POPs [31], although slightly higher than those obtained by a QuEChERS based method combined with comprehensive two-dimensional gas chromatography with time-of-flight mass

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spectrometry [32]. Compared to other published methods, the outstanding features and advantages of the procedure here presented are the use of a green extractant as well as the fully exploitation of the benefits of VALLME technique (speed, simplicity, efficiency, and high enrichment factor). The “greenness” of the present method was ascertained trough an Eco-scale as proposed by Gałuszka et al [33]. Therefore, for each parameter of the analytical method (amount of reagents, hazards, energy and wastes), penalty points are assigned if it departs from the 12 principles of green chemistry. As a result, the proposed method can be classified as “an excellent green analysis” since it scored more than 85 on the analytical Eco-scale (**Table S5)**. Additionally, the developed method easily allows the handling of 12 replicates at the same time taking around 20 min. of manual effort to finish a whole batch ready to inject. Overall, the current method allows reliable monitoring studies without compromising environmental health.

Real samples analysis

Aiming to demonstrate the applicability of the optimized DES-VALLME-GC-MS/MS method two fish oil samples for animal feed and three fish oil supplements for human consumption, rich in omega-3 fatty acids were analyzed as previous described. The levels found of the analytes studied are showed in **Table 4**.

Total levels of POPs were higher in fish oils for animal feed than those aimed to humans, with average levels of 21.1 and 8.9 ng g^{-1}, respectively. This can be explained by the more rigorous process applied to refined fat foodstuffs (neutralization, bleaching, and deodorization steps), that can probably reduce the levels of these kind of pollutants [17]. Despite the higher levels found in fish oils for animals, it should be stressed that also in fish oils for human consumption most of the contaminants (4,4 DDT, BDE47, BDE99, BDE153, BDE154) were found. This can be explained by the fact that removal rates are too much lower for some contaminants, such as 4,4 DDT or PBDEs [8,34].

It was also observed that both fish oils intended for animal consumption (Sardine_AF and Tuna_AF) had similar levels of OCPs and both were higher than those found on fish oil pills for human consumption (n3-HF1, n3-HF2 and n3-HF3), which is consistent with a previous 17]. In fish oil pills, only 4,4 DDT was quantified in one sample. The total levels of OCPs in the two samples for animal feed were 5.9 and 13 ng g^{-1}, with 4,4 DDT representing over 65% of this content in sardine fish oil. The levels of HCB and α-HCH in fish oil for animal feed were very similar, with averages of 6 ng g^{-1} and <LOQ, respectively. Similar results are reported by Nevado *et al.* [2] with 4,4 DDT showing

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higher levels than HCH and HCB in all three fish oil pills (omega-3 fatty acids supplements) analyzed.

Concerning to PBDEs, total levels were slightly higher in fish oils for animals than in human supplements. In both type of fish oils the PBDEs levels ranged from 1 to 6 ng g^{-1}, being BDE28 the less frequently detected. BDE153 levels were lower than BDE154 levels in all cases, ranging the ratio BDE153/BDE154 from 0.4 to 0.7 ng g^{-1}, which is in agreement with profiles reported in literature by other authors [1,9,17,32].

CONCLUSIONS

In the present study, a VALLME based on the use of a DES mixture as extractant followed by GC-MS/MS was developed for determination of the most relevant OCPs and PBDEs in fish oils used in human and animal supplements. Choline chloride – phenol (1:2 molar ratio) as DES showed good potential for extraction of different classes of POPs. A significant advantage of the proposed procedure is that only 180 µL of DES: EtOH (1:1 v/v) solution was used for each extraction, demonstrating to be an economical and environmentally friendly method with the elimination of both use and generation of hazardous substances, allowing to achieve an excellent ranking on the analytical Eco-Scale. Under the optimized conditions, the developed method provides a sub ng g^{-1} level of detection for all the analytes. The linearity was good in the range from 1 to 100 ng g^{-1}. The overall recoveries ranged from 76 to 90% with RSD values <20% and a measurement uncertainty value lower than 18.4%. The developed method was applied to analyze POPs in fish oil samples and it was demonstrated to be a practical and reliable method for monitoring studies.

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Figure 1. Total ion Chromatograms of the POPs extracted of a spiked olive oil (100ng g^{-1}) using different DESs (same scale) as solvent extractor in DLLME. Legend: A- ChCl-Gly, B- ChCl-Lac-1, C -ChCl-Lac-2, D- ChCl-Ur, E- ChCl-Ph-2, F- ChCl-Ph-3, G- ChCl-Ph-4, H- ChCl-Eg-2 and I- ChCl-Eg-3. Legend: Chromatogram of the five PBDE congeners and the three OCPs studied in this work at concentration 100 ppb. Also the internal standards, TPP and FBDE-12 are presented. (a) HCB and HCH, (b) 4,4.DDT (c) BDE-28 and TPP, (d) BDE-47, (d) BDE-99, (f) FBDE-126, (g) BDE-153, (h) BDE-154.

Figure 2- Total ion Chromatograms of the BDE47 extracted of a spiked olive oil (100ng g^{-1}) using A- ChCl-Ph-2 with methanol (1:1), B- ChCl-Ph-2 with ethanol (1:1) , A- ChCl-Ph-2

Figure 3- Response contour plot for DES volume (µL) and amount of oil (mg).

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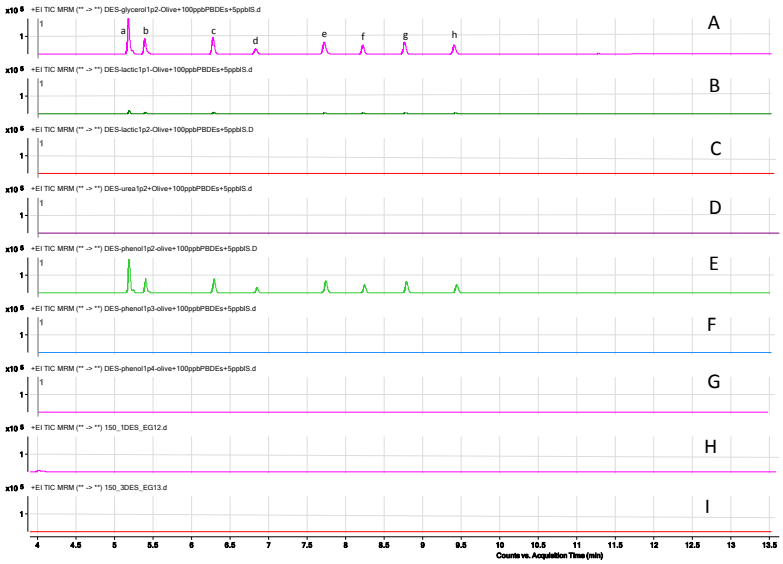

Figure 1

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Figure 2

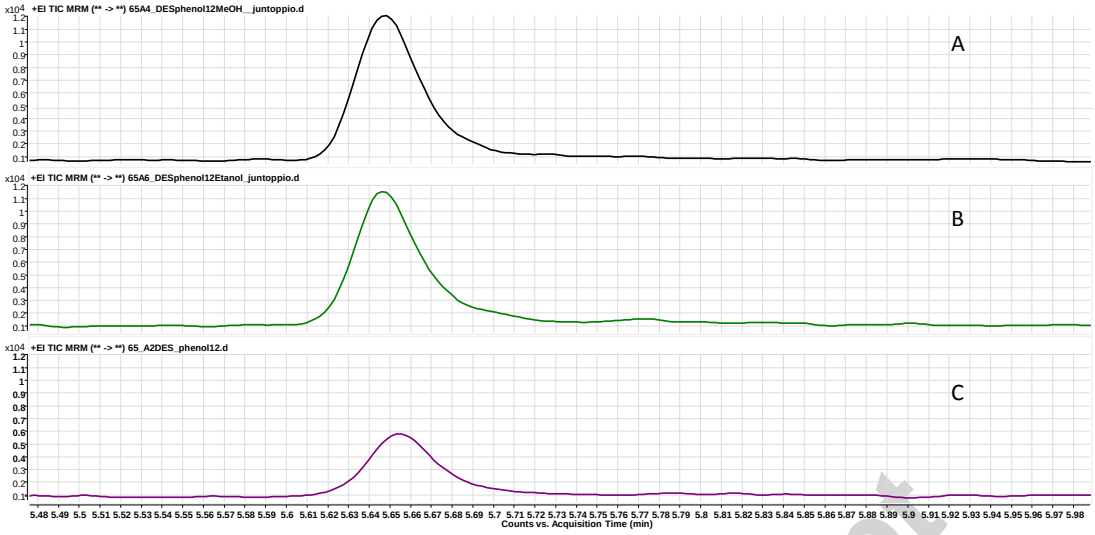

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Figure 3

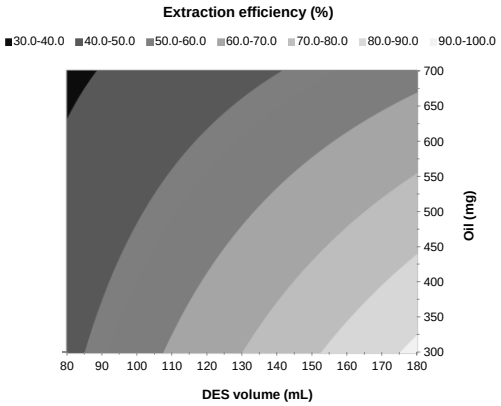

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Table 1- Different composition of DESs applied in this work.

HBA	HBD	Mole ratio	Abbreviation
Choline chloride	Phenol	1:2	ChCl-Ph-2
		1:3	ChCl-Ph-3
		1:4	ChCl-Ph-4
	Glycerol	1:2	ChCl-Gly
	Urea	1:2	ChCl-Ur
	DL-lactic acid	1:1	ChCl-Lac-1
		1:2	ChCl-Lac-2
	Ethylene glycol	1:2	ChCl-Eg-2
		1:3	ChCl-Eg-3

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Table 2- ANOVA table of varieties.

Source[a]	Sum of squares	df	Main square	F value	p-value[b]
A	1103.3	1	1103.3	17.59	0.0009
B	1924.6	1	1924.6	30.68	0.0001
C	5.97	1	5.97	0.1	0.7623
AB	359.3	1	359.3	5.73	0.0313
AC	118.6	1	118.6	1.89	0.1908
BC	2.16	1	2.16	0.03	0.8556
Total error	878.2	14	62.7		
Total (corr.)	5325.8	20			

[a] DES volume (A), amount of oil (B), extraction time (C).
[b] p < 0.05 indicates statistical significance

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Table 3- Summary of calibration curve, correlation coefficient, LOD and precision at three levels of ng/g

Analyte	Regression equation	Determination coefficient (r)	LOD ng/g	%RSD repeatability (%RSD intermediate precision)		
				5 ng/g	25 ng/g	75 ng/g
α-HCH	y = 0.0167x - 0.082	0.9970	0.5	11 (13)	6 (10)	2 (5)
HCB	y = 0.3135x - 0.8396	0.9968	0.4	1 (2)	3 (8)	18 (11)
4,4-DDT	y = 0.2085x - 0.031	0.9943	0.4	20 (14)	17 (12)	12 (16)
BDE-28	y = 0.1888x + 0.3926	0.9903	0.2	7 (5)	5 (5)	2 (4)
BDE-47	y = 0.1278x - 0.0963	0.9979	0.3	7 (10)	4 (7)	3 (9)
BDE-99	y = 0.0529x - 0.0443	0.9967	0.5	7 (15)	4 (11)	1 (9)
BDE-153	y = 0.0861x - 0.0617	0.9930	0.5	14 (15)	8 (10)	2 (8)
BDE-154	y = 0.0573x - 0.0453	0.9947	0.7	16 (12)	5 (11)	2 (9)

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Table 4. Concentration (in ng/g) of the PBDEs and OCPs in the five fish oils studied in this work. ND represents not detected

Analyte	Sardine_AF	Tuna_AF	n3-HF1	n3-HF2	n3-HF3
α-HCH	<LOQ	<LOQ	ND	ND	ND
HCB	5.9 ± 0.3	6.3 ± 0.4	ND	ND	ND
4,4-DDT	13.0	5.3 ± 2	8 ± 0.5	ND	ND
Total OCPs	**18.86**	**11.6**	**8**	**N**	**ND**
BDE28	ND	<LOQ	<LOQ	ND	ND
BDE47	1.1 ± 0.1	<LOQ	1.5 ± 0.2	ND	ND
BDE99	2.1± 0.4	2.3 ± 0.3	1.6 ± 0.2	ND	ND
BDE153	2.8 ± 0.2	2.9 ± 0.4	< LOQ	1.9 ± 0.04	2.8 ± 0.3
BDE154	1.6 ± 0.3	4.7 ± 0.2	< LOQ	4.6 ± 0.4	5.9 ± 0.3
Total PBDEs	**7.6**	**9.9**	**3.1**	**6.48**	**8.7**
Total POPs	***13.46***	***21.5***	***11.1***	***6.48***	***8.7***

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HIGHLIGHTS

- A green analytical method based on VALLME-GC-MS/MS was developed for POPs analysis
- DES (choline chloride:phenol) was used on VALLME as extractor solvent
- PBDES and OCPs were simultaneous analysed by GC-MS/MS
- The novel method was successfully applied to determine PBDEs and OCPs in fish oils

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