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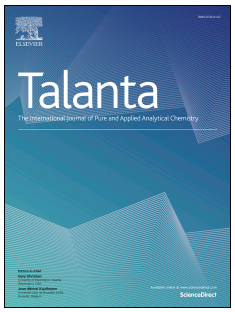

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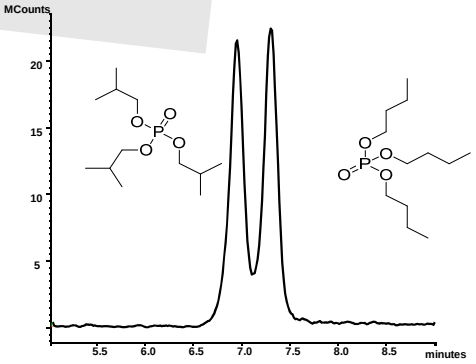
MSPD

LC-QqQ



DETERMINATION OF 18 ORGANOPHOSPHORUS FLAME RETARDANTS/PLASTICIZERS IN MUSSEL SAMPLES BY MATRIX SOLID-PHASE DISPERSION COMBINED TO LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Abstract

This study presents the development and validation of a new analytical method based on matrix solid-phase dispersion (MSPD), integrating sample extraction and clean-up in one single step, followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the simultaneous determination of 18 organophosphorus flame retardants and/or plasticizers (OPEs) in marine mussel (*Mytilus edulis* and *Mytilus galloprovincialis*) samples. Among these OPEs, 5 (tetraethyl 1,2-ethanediylbis(phosphonate), 6H-dibenzo[c,e][1,2]oxaphosphinine 6-oxide, tris(2,3-dibromopropyl) phosphate, 2,2-propanediyldi-4,1-phenylene bis(phosphate) and resorcinol bis(diphenyl phosphate)) are considered here for the first time in marine samples. Different parameters affecting the MSPD (clean-up sorbent and elution solvent) were optimized to obtain a good compromise between analyte recoveries and extract clean-up. Also, particular attention was paid to tackle blank issues. The overall method was validated in terms of trueness, precision and detection and quantification limits. Percentages of recovery varied from 69% to 122% with relative standard deviations below 24%. Detection limits ranged from 0.06 to 5 ng g^{-1} and quantification limits from 0.19 to 17 ng g^{-1} dry

weight. Finally, the method was applied to the analysis of 7 mussel samples collected in the coast of Galicia (Spain). 8 OPEs were detected in these samples at concentrations ranging from the LOQ to 291 ng g^{-1} dry weight.

Keywords: sample preparation, organophosphate esters, marine biota, mollusc bivalves

INTRODUCTION

Organophosphate esters (OPEs) are extensively used as flame retardants and plasticizers by the industry, their production having increased in the last years, since the use restrictions or ban of brominate flame retardants (BFRs) due to their confirmed persistence, bioaccumulation and/or toxicity [1]. There has also been a number of studies evaluating the effects of OPEs may have on human health and the environment in the last years, showing that OPEs could be potential carcinogens, endocrine disruptors and have neurotoxic effects [2]. In fact, the results obtained by Behl et al using the nematode *Caenorhabditis elegans* suggest that some aromatic OPEs (e.g. triphenyl phosphate – TPhP) may have levels of toxicity comparable to BFRs [3].

OPEs may be released from the plastic materials and diffuse into the environment, resulting into their frequent detection in various environmental matrices, such as water [4], sediment [5] and fish [6,7]. Also, their metabolites have been detected in human urine or in wastewater (due to urinary excretion) [8–10], thereby confirming the widespread human exposure to these chemicals through different routes [11]. The potential presence of OPEs in different environmental compartments and the growing list of studies which linked these compounds to significant health/ecotoxicological problems needs sensitive and selective methods for their determination, covering as many OPEs as possible.

Pollution of the marine environment because of human activity results in deleterious effects for the marine life, but also human health, via ingestion of marine seafood. In this context, mussels represent a potential human health issue, while they are also considered a good bioindicator of marine environmental quality, as they are filter-feeding organisms with a low capacity to eliminate toxic compounds. Furthermore, they have a wide geographic distribution and can be harvested from natural or farmed populations. Mussels have been used extensively in marine monitoring programs [12–14].

Several extraction methods based on pressurized liquid extraction [15], microwave assisted extraction [16], simple solid-liquid extraction by shaking [17] and high speed solvent extraction [18] have been used for sample preparation prior to the determination of OPEs in marine biota. After the extraction, usually, a clean-up step is necessary to eliminate co-extracted lipids and other interferents. In this framework, matrix solid-phase dispersion (MSPD) is an interesting sample preparation alternative, since extraction and clean-up are performed in a single step. In addition, MSPD reduces solvent consumption and has a low overall cost in comparison to classic sample preparation methods [19]. As regards instrumental analysis, gas chromatography (GC) coupled to MS [20] and especially liquid chromatography (LC) coupled to tandem MS (MS/MS) [21–23] are the most popular hyphenated techniques [24] for the determination of OPEs.

Hence, the aim of this study consisted of developing an extraction method based on MSPD for the simultaneous determination of 18 OPEs in mussel samples including 5 compounds not considered in previous studies in marine samples, using LC-MS/MS for separation and determination. Different parameters affecting the MSPD (e.g. amount and type of sorbents and solvents) were optimized to obtain a good compromise between analyte recoveries and extract clean-up. Finally, the developed method was validated in terms of trueness, precision and detection and quantification limits, and applied to the analysis of 7 mussel samples collected in the coast of Galicia (NW Spain).

EXPERIMENTAL

Standards and reagents

Tris(2-chloroethyl) phosphate (TCEP), tricresyl phosphate (TCrP), tris(2-butoxyethyl) phosphate (TBEP), tris(2-chloroisopropyl) phosphate (TCPP), tris(1,3-dichloro-2-propyl) phosphate (TDCP), triphenyl phosphate (TPhP), tri-n-butyl phosphate (TnBP), tris(2,3-dibromopropyl) phosphate

(TDBPP), tri-iso-butyl phosphate (TiBP), 2,2-propanediyldi-4,1-phenylene bis(phosphate) (BDP), 2-ethylhexyl-diphenyl phosphate (EHDPP) and tetraethyl 1,2-ethanediylbis(phosphonate) (TEEdP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tetrekis(2-chloroethyl)dichloroisopentyl diphosphate (V6), cresyl diphenyl phosphate (DCP), resorcinol bis(diphenyl phosphate) (RDP) and 6H-dibenzo[c,e][1,2]oxaphosphinine 6-oxide (DOPO) were purchased from Accustandard (New Haven, CT, USA), tripentyl phosphate (TPeP) from TCI Europe (Zwijndrecht, Belgium) and tris(2-ethylhexyl) phosphate (TEHP) from Wellington Laboratories (Guelph, Ontario, Canada). Five deuterium labelled OPEs were used as internal standards (TnBP-d27, TDCP-d15, TPhP-d15, TCEP-d12 and TCCP-d18) purchased from Wellington Laboratories.

Acetonitrile and methanol gradient grade for liquid chromatography solvents were provided by Merck (Darmstadt, Germany). Ultra-pure water was produced with a Milli-Q Gradient A-10 system (Millipore, Billerica, MA, USA).

Florisil (60-100 mesh) was provided by Supelco (Bellefonte, PA, USA), Bondesil-C18, 40 µm by Agilent Technologies (Santa Clara, CA, USA), alumina (150 mesh) by Sigma-Aldrich and silica gel 60 (0.040-0.063 mm) by Merck.

Samples

Mussels (*Mytilus edulis* and *Mytilus galloprovincialis*) were collected on several points along the northern coast of Spain during 2017. All samples were sent to our laboratory homogenized and freeze-dried in amber glass bottles by the Galicia Technological Institute for the Monitoring of the Marine Environment (INTECMAR). The amber glass bottles were stored into a box in a place with low humidity.

Precleaning of materials

In OPEs analysis, it is important to be particularly careful due to possible glassware and solvents contamination [24,25]. To minimize procedural blanks' contamination, cartridges and frits were sonicated in acetonitrile for 20 minutes in an ultrasonic bath. Silica and Florisil were washed in a PLE system using an ASE 200 (Dionex, Idstein, Germany) apparatus, equipped with 33 mL stainless steel extraction cells, using first acetonitrile and then ethyl acetate at 60 ° C. After that, sorbents were dried into the oven at 120 ° C for 24 hours. All glassware was washed with acetonitrile immediately before being used.

Sample preparation

Under optimal conditions, 0.5 g freeze-dried mussel was mixed with 1.2 g activated silica into a glass mortar. The homogeneous mixture was transferred into a cartridge containing 3 g of deactivated (5% H_20, w/w) Florisil. Then a frit was placed on top of the mixture and compressed. Analytes were eluted by gravity with 10 mL of acetonitrile into a Turbovap glass cell. The extract was concentrated approximately to ca. 0.5 mL into a Turbovap II nitrogen concentrator (Zymark, Hopkinton, MA, USA). The remaining volume was transferred into a vial and evaporated to dryness under a purified nitrogen stream. The dried extract was reconstituted in 100 µL of methanol and filtered with a GHP® 13 mm 0.2 µm Syringe filter membrane (Pall Corporation, Port Washington, NY, USA). Finally, the extract was transferred to a micro glass insert for injection into the LC-ESI-MS/MS system.

LC-ESI-MS/MS determination

OPEs were determined using a Varian (Walnut Creek, CA, USA) LC-MS/MS system. The LC instrument comprised two isocratic, high-pressure mixing pumps (Varian ProStar 210), an autosampler and a thermostated compartment for the column (Varian ProStar 410). The mass spectrometer was a triple quadrupole (Varian 320-MS) furnished with an ESI interface. Instrument control and data acquisition were performed with the Varian MS Workstation 6.9.2 software. Chromatographic separation was carried out with a Luna 3 µm C18 column (50 x 2 mm) connected to a C18 (2 × 4 mm) guard cartridge,

both supplied by Phenomenex (Torrance, CA, USA). A 10 µL aliquot of the sample extract or standard was injected in the micro pick-up injection mode. As mobile phases, Milli-Q water (0.1 % formic acid) (A) and methanol (0.1 % formic acid) (B) were used at a flow rate of 0.2 mL/min and the temperature of the column was fixed at 35°C. The gradient elution started with 65% B, increasing to 100% B in 24 min, held for 2 min. Subsequently, it returned to initial conditions (65% B) in 0.1 min, held for 5 min for column back-conditioning.

MS determination was performed with nitrogen as nebulizing (55 psi) and drying gas (200 °C, 18 psi) in the ESI source, provided by a high purity generator (Domnick Hunter, Durham, UK). The voltage of the ESI needle was fixed at 5,000 V. The temperature of the ESI housing was set at 55 °C. Argon (99.999%) was employed as collision gas (2.2 mTorr) in the mass spectrometer. Analyses were performed in positive ion mode and compounds recorded in the multiple reaction monitoring (MRM) mode, using two transitions per compound and a dwell time of 50 ms per transition. The most intense transition was used for quantification and the second one for confirmation, as detailed in Table 1. The criteria for analyte positive identification in the samples was based on the transition's ratio, which should not differ more than a 30% from calibration standards, in accordance with the SANTE/11813/2017 guideline.

RESULTS AND DISCUSSION

LC-ESI-MS/MS

Optimization of ESI-MS/MS parameters was performed by direct infusion of 10 µg mL^{-1} individual standards of each compound in methanol. The optimal detection conditions are shown in Table 1. The chromatographic conditions described in the LC-ESI-MS/MS determination section were based

on a previous work [26], obtaining a good chromatographic separation for the 18 OPEs, including the isomers TiBP and TnBP.

Instrumental performance parameters are summarized in Table 1. Linearity was determined by injecting seven different concentration levels of standards in the iLOQ-500 ng mL^{-1} range (IS at 50 ng mL^{-1}), generating determination coefficients (R^2) between 0.9982 and 0.9999. Detection and quantification limits (iLODs and iLOQs) were estimated for a signal-to-noise (S/N) ratio of 3 and 10, respectively, using a standard at low concentration level. In general terms, iLODs were lower than 0.5 ng mL^{-1} (iLOQs ≤ 1 ng mL^{-1}), except for V6 (iLOD was 10 ng mL^{-1}) and TDBPP (iLOD was 16 ng mL^{-1}), whose ionization by ESI was less efficient. The precision (repeatability and intermediate precision), expressed as relative standard deviations (RSD, %), was evaluated at two concentration levels: 10 and 100 ng mL^{-1}. For V6 and TDBPP precision could not be estimated at the lowest concentration level since the iLOQ is higher than 10 ng mL^{-1}. The RSD values for repeatability (n=6) were equal or lower than 13% (low level) and 9% (high level). The RSD values for intermediate precision (n= 18), estimated in three different days, were equal to or lower than 16% for all analytes at both concentrations.

MSPD optimization

Selection of clean-up sorbent and elution solvent

The procedure developed by Campone et al. [6] for the extraction of 13 OPEs from fish tissue was initially tested for the extraction of the 18 OPEs selected in this work from freeze-dried mussels. Briefly, 0.5 g of sample (spiked with 100 ng g^{-1} of the 18 OPEs) were mixed with 1 g anhydrous sodium sulphate, dispersed using 2 g of Florisil, and 1 g of alumina as co-sorbent at the bottom and the analytes eluted using 10 mL n-hexane/acetone (6:4 v/v) [6]. Good recoveries were obtained for 14 of the OPES, however for V6, DOPO, TDBPP and TEEdP, low recoveries, below to 20%, were obtained. So, a new MSPD procedure was developed. As starting point, 0.5 g of mussel and 1.2 g

169 silica as solid support (dispersing agent) were chosen. Internal standards were not employed during
170 method optimization.

171 Three different sorbents, one reversed-phase sorbent (C18) and two normal-phase sorbents (alumina
172 and Florisil), were evaluated as clean-up sorbent. Extractions (n=3) were carried out on spiked
173 samples (100 ng g^{-1} of the 18 OPEs), 3 g of clean-up sorbent in the MSPD column and 10 mL of either
174 ethyl acetate or acetonitrile as elution solvent. The collected extracts were concentrated to dryness
175 under a nitrogen stream and the dried extracts were reconstituted in 100 µL of methanol and filtered
176 through a GHP membrane. Recoveries obtained for the OPEs with each sorbent are shown in Fig. 1.
177 As it can be observed, low recoveries were obtained with the three sorbents for TEEdP, TDBPP and
178 DOPO, when ethyl acetate was used as eluent. Furthermore, for TEEdP, significantly higher
179 recoveries were obtained using acetonitrile with all the considered sorbents. On the other hand, for
180 DOPO and TDBPP, the combination of Florisil with acetonitrile provided the best recoveries. Under
181 these conditions, good recoveries were also obtained for the other 15 OPEs.

182 As regards the clean-up efficiency, the dry residue (mostly lipids) in the extract was evaluated
183 gravimetrically and expressed as the percentage referred to the freeze-dried mussel sample weight.
184 Ethyl acetate produced extracts with higher dry residue (C18: 1.3 ± 0.2 %; alumina: 0.72 ± 0.06 %;
185 Florisil: 0.84 ± 0.01 %) than acetonitrile (C18: 0.37 ± 0.04%; alumina: 0.31 ± 0.07%; Florisil: 0.49 ±
186 0.05%). Therefore, acetonitrile and Florisil were chosen as elution solvent and clean-up sorbent,
187 since this combination provides good recoveries and a relatively good clean-up.

188 **Procedural blanks**

189 Once the clean-up sorbent and elution solvent were selected a study of procedural blanks was
190 performed (n=3). Eight analytes, i.e. TCPP, TCEP, TBEP, TiBP, TnBP, EHDPP, TEHP and TPhP, were
191 found in the procedural blanks. In order to improve this situation, solid support (silica) and clean-up
192 sorbent (Florisil) were washed in a PLE system and all materials (glass and plastic) were washed just

193 before being used as described in the Precleaning of materials section. With this clean-up protocol,
194 TCPP, TBEP, EHDPP, TiBP and TnBP were still detected in the procedural blanks, but with a significant
195 reduction of their amount (Figure 2). In fact the concentration in the extract were lowered to levels
196 close to the iLOQs for many of them, viz.: TCPP: from 116 to 3 ng mL^{-1}, TBEP: from 10 to 0.5 ng mL^{-1},
197 EHDPP: from 47 to 0.5 ng mL^{-1}, TiBP from 86 to 2 ng mL^{-1} and TnBP from 27 to 1 ng mL^{-1}.

198 **Florisil deactivation**

199 As a result of the sorbents cleaning process, Florisil suffers an activation process which can lead to
200 excessive sorbent-analyte interaction. Deactivation of sorbents with water is frequently performed
201 [27] in order to control the water content and therefore analytes recoveries and clean-up. The
202 degree of deactivation is specified by the weight percent of water added to the sorbent. Three
203 percentages (w/w) of Milli-Q water were tested: 0, 5 and 10%. As shown in Figure 3, 5% deactivated
204 Florisil provided better recoveries than activated Florisil and 10% deactivated Florisil. In terms of fat
205 content (n=3), similar clean-up was obtained under the three working conditions, activated Florisil
206 (0.34 ± 0.03%), 5% deactivated Florisil (0.39 ± 0.01%) and 10% deactivated Florisil (0.31 ± 0.03%)
207 were reached. Therefore, Florisil deactivated with 5 % of Milli-Q water was selected as clean-up
208 sorbent material.

209 ## Analytical performance of the developed method

210 **Matrix effects**

211 Matrix effects (ME) produced by co-eluting matrix components were evaluated by comparing mussel
212 extracts (n=3) spiked over the extract with 100 ng mL^{-1} of compounds with standards at the same
213 concentration level and expressed as % relative response after subtracting non-spiked responses. In
214 this way, values of ME higher than 100% indicate a signal enhancement, lower than 100% a signal
215 suppression and 100% no matrix effects [28]. As shown in Figure 4, for most compounds a significant

signal suppression was observed, especially for TCEP (ME=18 %), TDBPP (ME=23 %) and TDCP (ME=28 %). In the case of TiBP, TnBP and TBEP, the values of matrix effects were around 100%. BDP is the only compound that exhibited a moderate signal enhancement (117 %). These matrix effects were compensated using five internal standards available in the laboratory except for BDP (see section below).

Trueness, precision and limits of detection and quantification

The performance figures of the proposed method are summarized in Table 2. Trueness and precision were calculated for spiked mussel samples at two levels, 10 and 100 ng g^{-1} of the studied OPEs, containing 10 ng g^{-1} IS in all cases. Four replicates of spiked mussel samples and three replicates of the non-spiked samples were performed. Internal standard calibration, with the IS indicated in Table 1, was used for quantification purposes, except for BDP. For this compound the signal enhancement could not be compensated using any IS and unfortunately, its isotopically labelled analogue was not commercially available. So, the standard addition method over the extract was used for BDP quantification. For TCEP, TDBPP, TDCP, TEEdP and V6, trueness and precision could not be estimated at the lowest level since the mLOQs are higher than 10 ng g^{-1}. At this lowest concentration level (10 ng g^{-1}), recovery values varied between 82% for DOPO and 117% for RDP. At the highest level (100 ng g^{-1}), recovery values varied between 69% for TEEdP and 122% for V6. The precision, expressed as % RSD, was below 24 % and 9 % for 10 ng g^{-1} and 100 ng g^{-1}, respectively.

Method detection and quantification limits (mLODs and mLOQs) were calculated with the same method used to estimate the iLODs and iLOQs, using the lowest level spiked mussel sample. For those compounds present in the procedural blank, the mLOD and mLOQ were also estimated by multiplying by 3 and 10 the standard deviation of the signal in the procedural blank (n=3), respectively. For these compounds, from the two estimation methods, the one that provided the highest mLODs and mLOQs was selected. mLODs ranged from 0.06 to 5 ng g^{-1} and mLOQs from 0.19

to 17 ng g^{-1}. To the best of our knowledge, this is the first method developed for the determination of OPEs in mussel samples. However, some methods have been published for the analysis of marine biota, mainly fish samples. For comparison purposes, mLODs were also referred to lipid weight (lw), considering an average humidity of 85 % and an average fat content of 7 %. In this way, mLODs would range from 0.1 to 11 ng g^{-1} lw. Table 3 compares these results to those published in the literature for other marine biota species. Similar or lower mLODs than those reported in the literature [6,22,29] were obtained for most of the compounds, except for TCEP and TDCP (Table 3) which present slightly higher mLODs. There is also another method published by Liu et al. [30], but it does not provide units for the mLODs they published, thus, it is not considered in Table 3. Five out of the 18 compound studies (TEEdP, DOPO, TDBPP, RDP and BDP) had not been included in the already published methods, therefore, a comparison cannot be performed.

Analysis of real samples

The developed method was applied to seven mussel samples collected along the coast of Galicia (NW Spain). During the analysis process, three procedural blanks were performed together with each sample batch and then blank concentrations were subtracted from the sample concentrations. 8 OPEs were detected in these samples at concentrations ranging from the LOQ to 291 ng g^{-1} dw (Table 4). Among them, TBEP and TPhP were found at concentrations above the mLOQ in all the samples tested, followed by TCPP (6 samples), TiBP (5 samples), TEHP and TnBP (4 samples) and EHDPP (2 samples). TCEP was detected but at concentrations below the mLOQ. In terms of concentration, TPhP was the analyte detected at higher levels (11-291 ng g^{-1} dw), far higher than the remaining OPEs. As an example, Figure 5 depicts the chromatograms of sample F. Alvarez-Muñoz et al. found also TBEP and TCEP in all the samples at concentrations ranging from 7.1 ng g^{-1} dw to 39.4 ng g^{-1} dw for TBEP, and below the mLOQ for TCEP [31]. If we compare the concentrations found in mussels in this work

to those reported in the literature for fish samples, once converted to lipid weight basis (compiled in Table 5), they are at the same order of magnitude.

CONCLUSIONS

A method for the comprehensive determination of 18 OPEs in mussel samples, including analytes that had not even been considered in previous marine biota studies, has been developed. The optimized MSPD method provides a good compromise between extraction and clean-up in a fairly simple and rapid protocol. The method was validated with satisfactory results reaching mLODs between 0.06 and 5 ng g^{-1} (0.1-11 ng g^{-1} lw). Its application to the analysis of 7 mussel samples showed the presence of eight OPEs at concentrations ranging from the LOQ to 291 ng g^{-1} dw, which represent values similar to those already reported in fish samples.

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Figure captions

Figure 1: % Relative recoveries (normalized to the highest value) obtained with different sorbents and solvents for mussel samples spiked at 100 ng g^{-1} (n = 3). ACN: acetonitrile; AcOEt: ethyl acetate.

Figure 2: Chromatograms of the compounds found in the procedural blanks without (dotted line) and after the clean-up protocol described in the Precleaning of materials section (solid line).

Figure 3: Effect of the amount of water used to deactivate Florisil on analytes response (n = 3). Values normalized to the highest response.

Figure 4: Matrix effects obtained with mussel extracts spiked at 100 ng mL^{-1} over the extract (n = 3).

Figure 5: Chromatograms of the compounds detected in Sample F.

Table 1: LC-ESI -MS/MS experimental parameters (CV: capillary voltage, CE: collision energy) and instrumental performance figures.

Compound	t_R (min)	Quantification transition (m/z) (CE, V)	Qualifier transition (m/z) (CE, V)	CV (V)	IS	Linearity (R^2)[a]	Repeatability % RSD (n=6)[b]		Intermediate precision % RSD (n=18)[c]		iLOD (ng mL^{-1})	iLOQ (ng mL^{-1})
							10 ng mL^{-1}	100 ng mL^{-1}	10 ng mL^{-1}	100 ng mL^{-1}		
TEEdP	1.6	303>173 (24.0)	303>109 (41.5)	44	TDCP-d15	0.9997	4.2	2.0	6.3	5.7	0.02	0.08
DOPO	1.7	217>199 (19.0)	217>152 (32.0)	84	TCPP-d18	0.9998	2.8	2.4	6.0	5.6	0.06	0.2
TCEP	1.8	285>223 (9.5)	285>99 (20.0)	52	TCEP-d12	0.9997	4.7	3.7	7.9	5.4	0.2	0.5
TCPP	3.3	327>99 (20.0)	327>251 (7.5)	44	TCPP-d18	0.9998	5.4	1.6	5.7	3.0	0.4	1
V6	3.4	605>361 (22.5)	607>361 (23.5)	100	TPhP-d15	0.9982	< iLOQ	6.8	< iLOQ	9.2	10	35
TDCP	5.5	431>99 (21.0)	433>99 (22.0)	56	TDCP-d15	0.9983	13.1	6.0	15.9	6.6	0.1	0.5
TPhP	5.6	327>152 (33.5)	327>215 (22.5)	80	TPhP-d15	0.9996	5.6	3.7	5.3	5.0	0.1	0.5
TDBPP	6.7	699>99 (19.0)	699>299 (14.5)	60	TDCP-d15	0.9994	< iLOQ	8.6	< iLOQ	7.5	16	55
TiBP	6.9	267>99 (14.0)	267>155 (7.0)	48	TnBP-d27	0.9993	3.1	2.9	3.1	2.5	0.1	0.3
DCP	7.2	341>229 (22.0)	341>165 (28.5)	104	TnBP-d27	0.9993	10.6	4.6	13.0	7.3	0.1	0.4
TnBP	7.2	267>99 (14.0)	267>155 (7.0)	48	TnBP-d27	0.9999	3.3	3.2	4.4	4.0	0.08	0.2
TBEP	8.6	399>199 (11.5)	399>299 (9.5)	64	TnBP-d27	0.9995	7.4	2.7	5.4	4.6	0.1	0.4
RDP	10.3	575>215 (45.0)	575>481 (33.0)	136	TPhP-d15	0.9983	13.2	2.4	13.8	8.8	0.01	0.05
TCrP	10.7	369>165 (45.0)	369>243 (24.5)	92	TPhP-d15	0.9998	4.2	3.5	5.2	4.3	0.04	0.1
EHDPP	12.2	363>251 (5.5)	363>152 (35.5)	48	TnBP-d27	0.9982	9.6	2.4	8.6	7.3	0.09	0.3
TPeP	12.7	309>99 (15.5)	309>239 (6.5)	44	TCPP-d18	0.9999	3.9	2.4	4.0	3.5	0.04	0.1
BDP	15.6	693>367 (27.5)	693>327 (21.5)	144	-	0.9990	8.8	1.0	8.8	7.4	0.02	0.06
TEHP	22.1	435>99 (11.5)	435>323 (5.0)	48	TCPP-d18	0.9991	4.2	3.6	9.7	15.2	0.04	0.2
TCEP-d12	1.7	297>102 (18.5)	297>232 (8.5)	52								
TCPP-d18	3.1	347>102 (17.5)	345>102 (17.5)	40								
TDCP-d15	5.3	446>102 (18.5)	448>102 (19.0)	56								
TPhP-d15	5.3	342>223 (20.5)	342>160 (30.5)	88								
TnBP-d27	6.9	294>102 (13.5)	294>106 (7.5)	40								

[a] Linear range iLOQ-500 ng mL^{-1} (IS: 50 ng mL^{-1}) [b] measured along the same day [c] measured over three different days

Table 2: Recoveries, repeatability (as %RSD, in brackets), detection (mLOQs) and quantification limits (mLOQs) of the MSPD-LC-MS/MS method. Analytes quantified by internal standard calibration, except BDP, which was quantified by standard addition over the extract.

Compound	Recovery (%) (RSD) (n=4)		mLOD	mLOQ
	10 ng g^{-1}	100 ng g^{-1}	(ng g^{-1}) dw	(ng g^{-1}) dw
TEEdP	< mLOQ	69 (9)	4	14
DOPO	82 (5)	101 (7)	0.8	2
TCEP	< mLOQ	86 (3)	4	14
TCPP	109 (1)	100 (4)	0.4	1
V6	< mLOQ	122 (8)	3	11
TDCP	< mLOQ	94 (9)	5	17
TPhP	100 (5)	103 (5)	0.3	0.8
TDBPP	< mLOQ	104 (6)	4	14
TiBP	109 (6)	98 (6)	0.2	0.7
DCP	99 (5)	116 (5)	2	5
TnBP	104 (5)	100 (3)	0.1	0.4
TBEP	85 (24)	93 (4)	0.3	1
RDP	117 (9)	110 (6)	0.2	0.6
TCrP	101 (7)	115 (6)	0.4	1
EHDPP	85 (12)	98 (8)	0.4	1
TPeP	94 (7)	88 (2)	0.08	0.3
BDP	90 (5)	99 (1)	0.06	0.2
TEHP	91 (9)	92(7)	0.1	0.5

Table 3: Comparison of mLODs obtained in this work to those from the literature where other marine biota is analysed.

	mLOD (ng g^{-1} lw)			
Compound	this work	[6]	[29]	[22]
TEEdP	9	NS	NS	NS
DOPO	2	NS	NS	NS
TCEP	9	0.4	1.2	1.4
TCPP	0.8	1	1.5	1.7
V6	7	NS	NS	4.7
TDCP	11	9	0.2	0.3
TPhP	0.6	0.8	1.3	6.4
TDBPP	9	NS	NS	NS
TiBP	0.4	0.2	NS	NS
DCP	3	NS	1.6	11.6
TnBP	0.2	0.2	3.4	37.4
TBEP	0.7	2.2	NS	0.8
RDP	0.4	NS	NS	NS
TCrP	0.8	3.1	2.5	NS
EHDPP	1	NS	0.5	0.4
TPeP	0.2	1.4	NS	NS
BDP	0.1	NS	NS	NS
TEHP	0.3	1.4	2.0	NS

NS: no studied

Table 4: Concentration (ng g^{-1} dw ± standard deviation) of OPEs detected in the analysed mussel samples (n=3). N.B.: those compounds which were <mLOD in all samples are not presented in the table.

Compound	Sample A [a] (Cee)	Sample B [b] (Ferrol)	Sample C [b] (A Coruña)	Sample D [b] (Arousa)	Sample E [a] (A Coruña)	Sample F [a] (Ferrol)	Sample G [a] (A Coruña)
EHDPP	< mLOQ	ND	ND	ND	2.0 ± 0.3	1.9 ± 0.1	< mLOQ
TBEP	1.96 ± 0.04	5.7 ± 0.5	5.7 ± 0.9	5.8 ± 0.7	5.7 ± 0.9	3.3 ± 0.5	4.9 ± 0.4
TCEP	ND	ND	ND	ND	ND	< mLOQ	< mLOQ
TCPP	13.8 ± 0.6	ND	1.85 ± 0.08	1.5 ± 0.5	4 ± 1	2.5 ± 0.4	1.3 ± 0.5
TEHP	2.0 ± 0.1	ND	ND	1.2 ± 0.2	ND	1.9 ± 0.2	1.01 ± 0.01
TPhP	13.3 ± 0.5	12 ± 1	13 ± 2	11 ± 1	291 ± 20	40 ± 6	71 ± 7
TiBP	ND	1.0 ± 0.1	1.3 ± 0.2	4.5 ± 0.4	7.1 ± 0.2	< mLOQ	1.0 ± 0.2
TnBP	ND	< mLOQ	2.6 ± 0.4	4 ± 1	4.4 ± 0.2	< mLOQ	1.4 ± 0.4

ND: not detected

[a]: Mytilus galloprovincialis

[b]: Mytilus edulis

Table 5: Concentration range of OPEs (ng g^{-1} lw) obtained in this work for mussel samples and those reported in the literature for marine and river fish samples.

	this work	[6]	[29]	[22]	[30]	[31] [b]
Samples	Mussel	Salmon and cod	Menida, Marble, trout and salmon	Barbel, carp and trout	Plecostomus, tilapia, mud carp and catfish	Clam, oyster and mussel
TEEdP	ND	NS	NS	NS	NS	NS
DOPO	ND	NS	NS	NS	NS	NS
TCEP	< mLOQ	ND	mLOQ - 10 [a]	8.6-134	6.11-19.5	<LOQ
TCPP	3.8-29.6	ND	mLOQ - 10 [a]	ND	23.5-28.9	ND
V6	ND	NS	NS	ND	NS	NS
TDCP	ND	ND	ND	ND	3.79	NS
TPhP	23.6-623.6	ND	mLOQ-25 [a]	ND	16.3-85	NS
TDBPP	ND	NS	NS	NS	NS	NS
TiBP	2.1-15.2	ND	NS	NS	NS	NS
DCP	ND	NS	mLOQ-10 [a]	ND	NS	NS
TnBP	0.9-9.4	ND	NS	ND	11.7-94.6	NS
TBEP	5.6-12.4	ND	10.5-209	6.4-296	1.19-22.9	15.2 - 85
RDP	ND	NS	NS	NS	NS	NS
TCrP	ND	ND	NS	NS	8.71-10.3	NS
EHDPP	4.1-4.3	NS	mLOQ-100 [a]	82.4-574	4.26-7.25	NS
TPeP	ND	ND	NS	NS	NS	NS
BDP	ND	NS	NS	NS	NS	NS
TEHP	0.2-4.3	ND	mLOQ-30 [a]	37-314	12.7-96.1	NS

NS: not studied; ND: no detected

[a] These values were obtained by visual estimation from Figure 3 presented in that article

[b] For comparison purposes, concentrations reported in the paper expressed as ng g^{-1} dw were referred to ng g^{-1} lw, considering an average humidity of 85 % and an average fat content of 7 %.

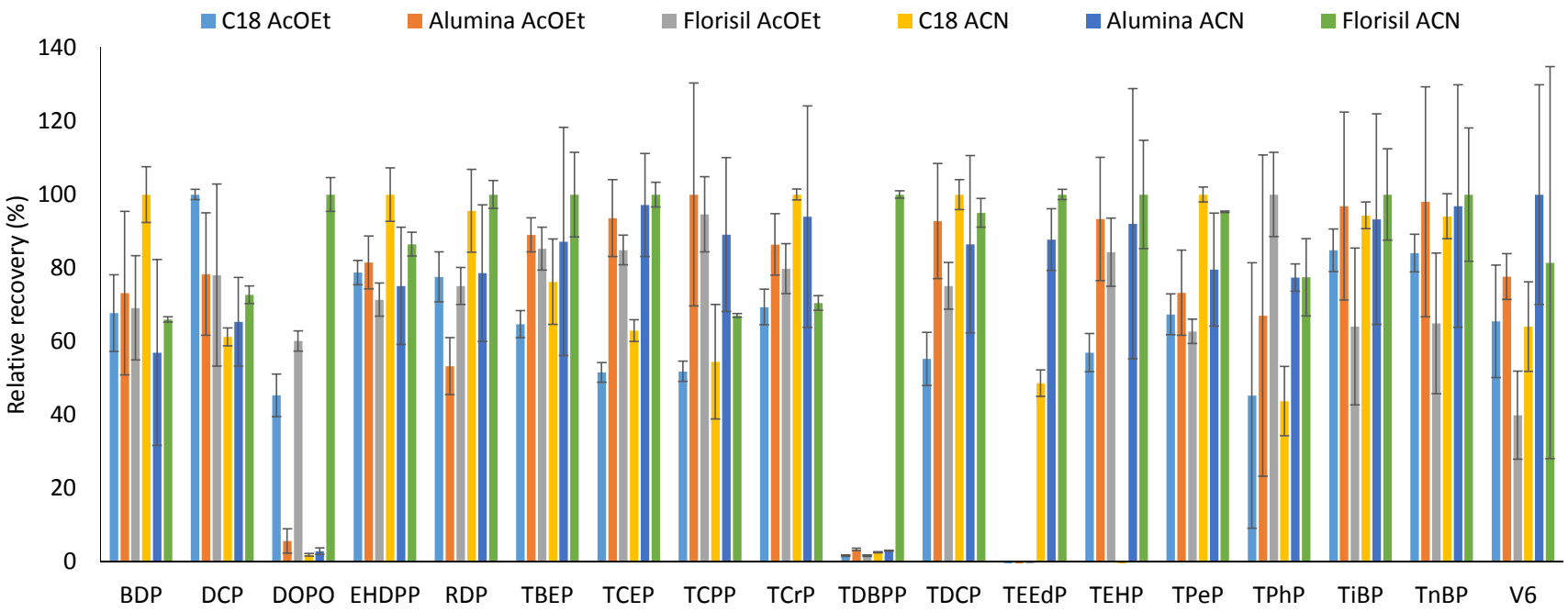

C18 AcOEt
Alumina AcOEt
Florisil AcOEt
C18 ACN
Alumina ACN
Florisil ACN
Relative recovery (%)
140
120
100
80
60
40
20
0
BDP
DCP
DOPO
EHDPP
RDP
TBEP
TCEP
TCPP
TCrP
TDBPP
TDCP
TEEdP
TEHP
TPeP
TPhP
TiBP
TnBP
V6

Figure 1

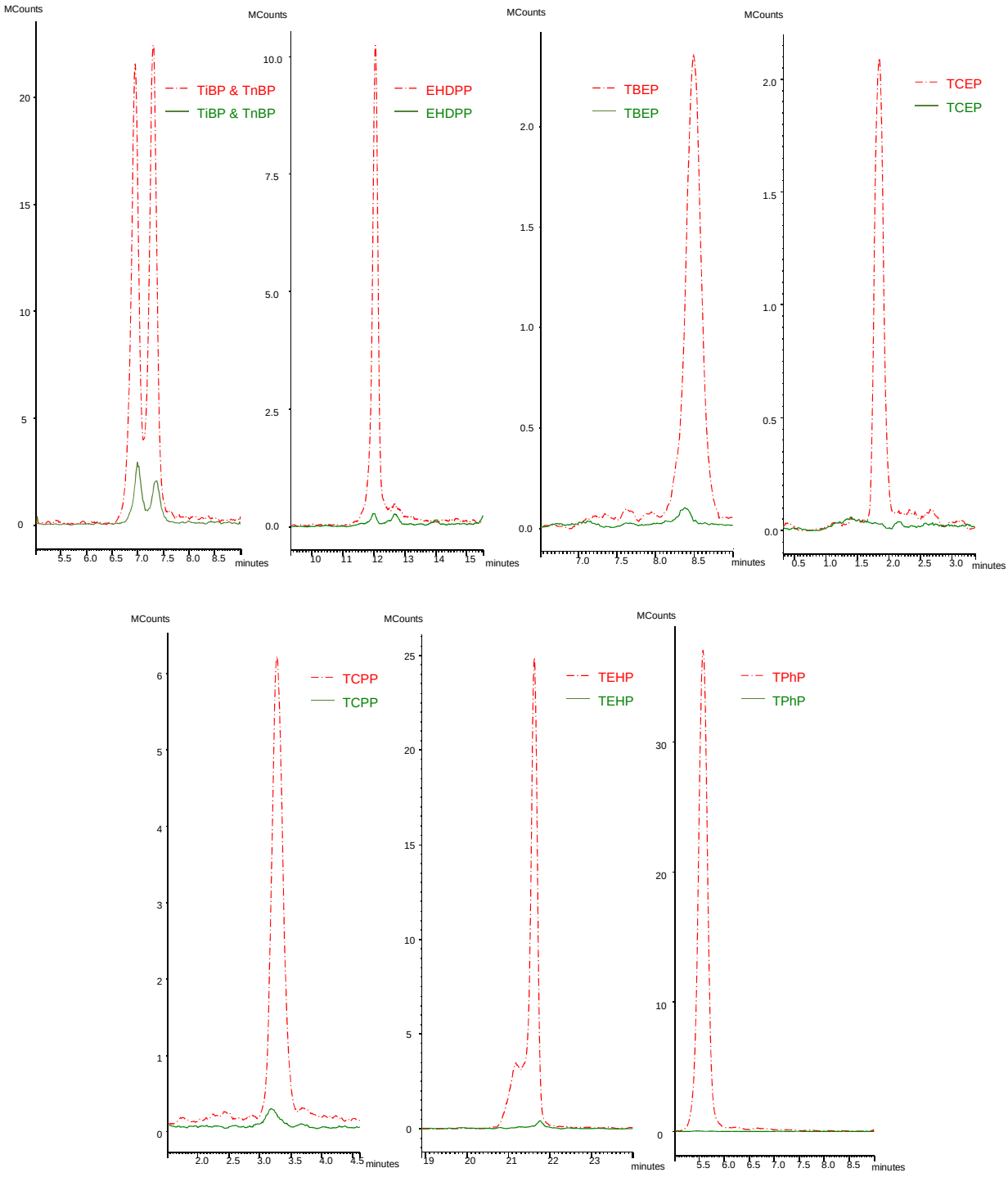

MCounts
TiBP & TnBP
TiBP & TnBP
minutes
MCounts
EHDPP
EHDPP
minutes
MCounts
TBEP
TBEP
minutes
MCounts
TCEP
TCEP
minutes
MCounts
TCPP
TCPP
minutes
MCounts
TEHP
TEHP
minutes
MCounts
TPhP
TPhP
minutes

Figure 2

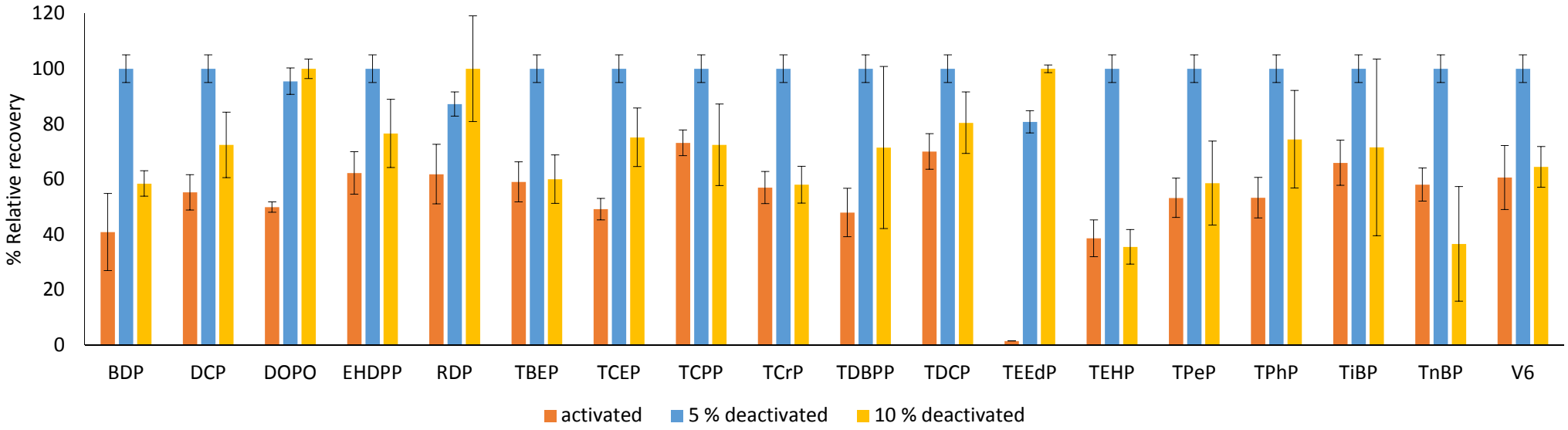
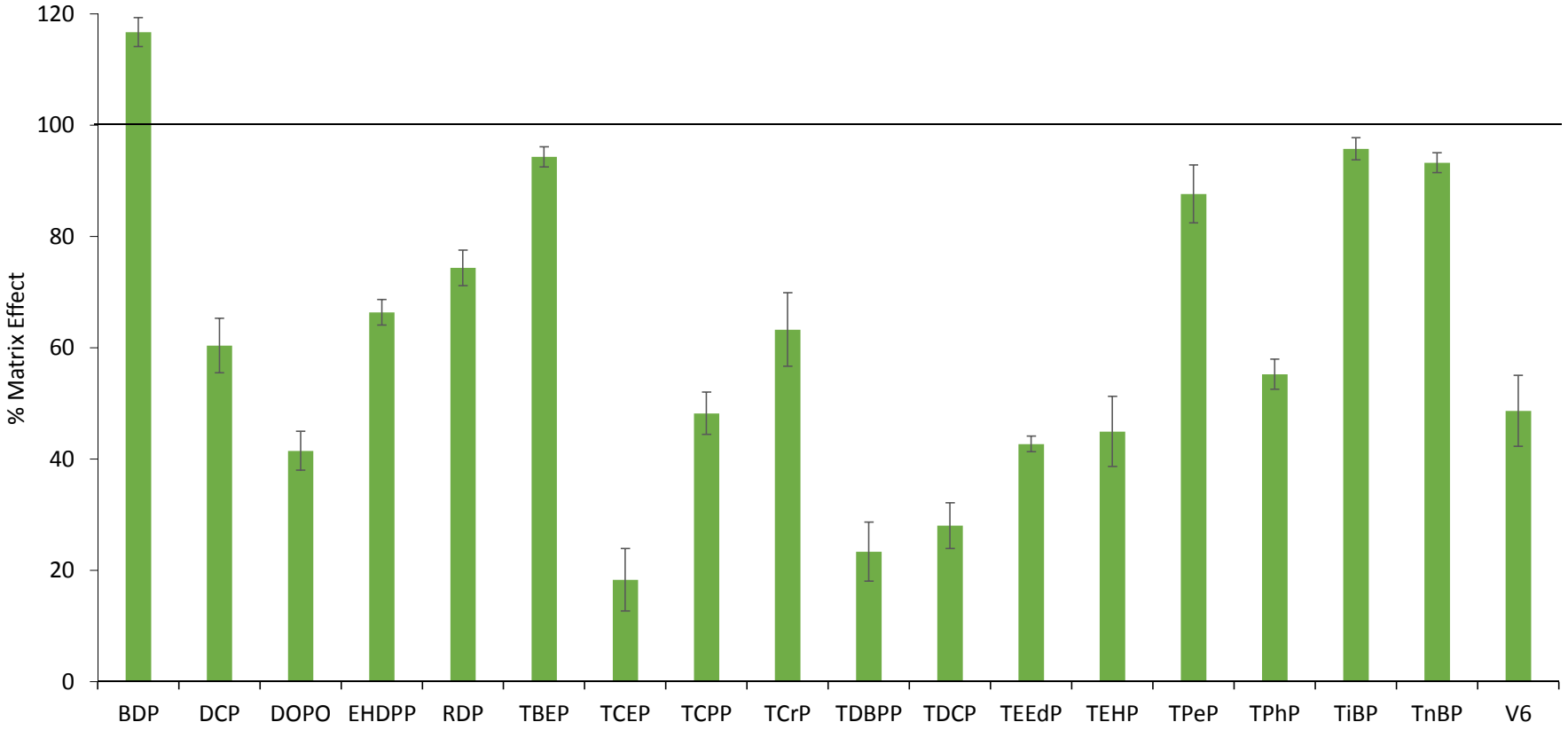

120
100
80
60
40
20
0
% Relative recovery
BDP
DCP
DOPO
EHDPP
RDP
TBEP
TCEP
TCPP
TCrP
TDBPP
TDCP
TEEdP
TEHP
TPeP
TPhP
TiBP
TnBP
V6
activated
5 % deactivated
10 % deactivated

Figure 3


120
100
80
60
40
20
0
% Matrix Effect
BDP
DCP
DOPO
EHDPP
RDP
TBEP
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TEHP
TPeP
TPhP
TiBP
TnBP
V6

Figure 4

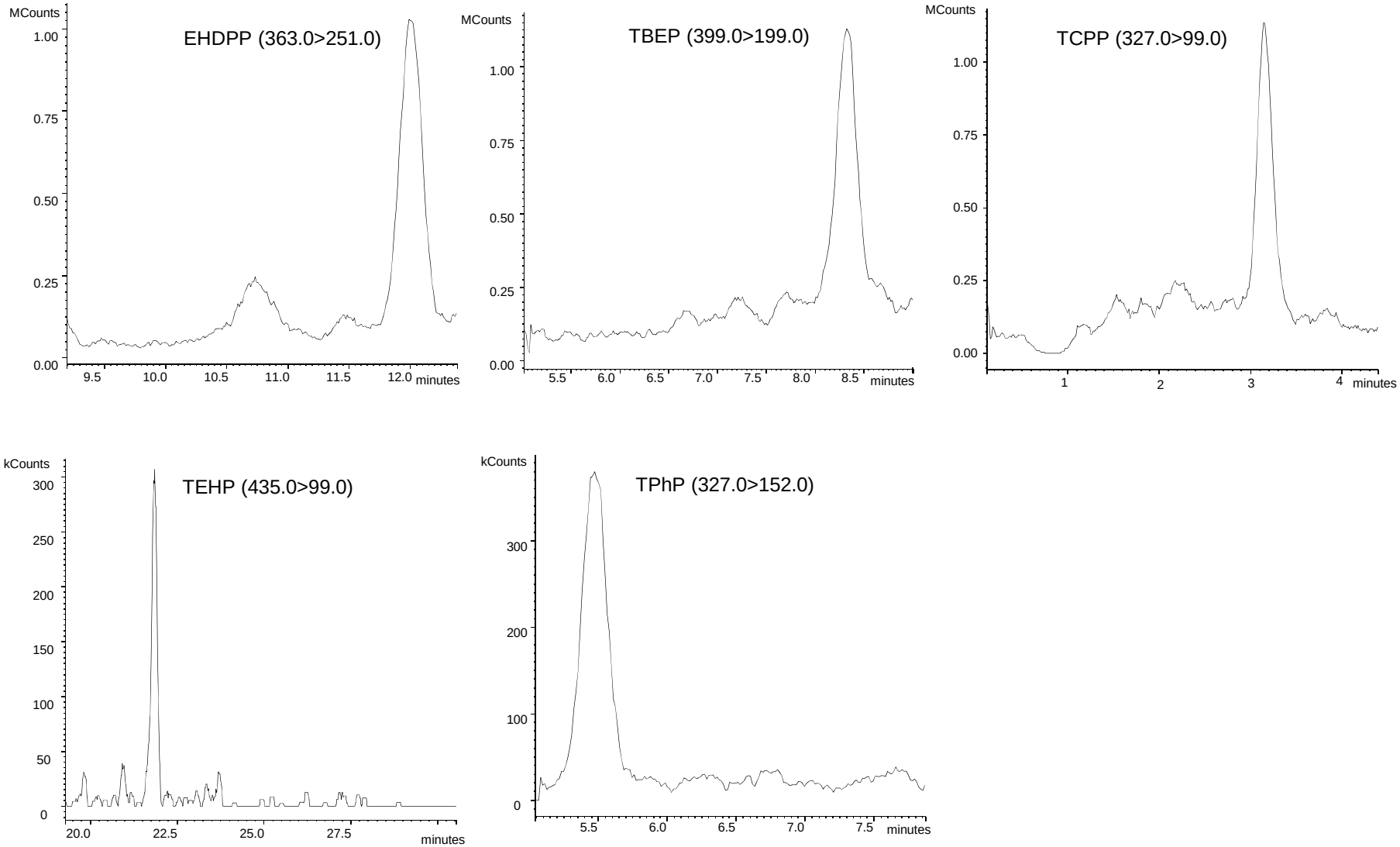
MCounts
EHDPP (363.0>251.0)
TBEP (399.0>199.0)
TCPP (327.0>99.0)
kCounts
TEHP (435.0>99.0)
TPhP (327.0>152.0)
minutes

Figure 5

Highlights

- Simple method for the determination of 18 OPEs in mussel samples
- 5 OPEs considered for the first time in marine samples
- Detection limits in the 0.06-5 ng g^{-1}dry weight range
- 8 OPEs were found in the mussel samples analysed
- Triphenyl phosphate detected in all samples up to 291 ng g^{-1} dry weight

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: