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Determination of persistent and mobile organic contaminants (PMOCs) in water by mixed-mode liquid chromatography-tandem mass spectrometry.

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Abstract

The presence of persistent and mobile organic contaminants (PMOC) in aquatic environments has become a matter of concern due to their ability of breaking through natural and anthropogenic barriers, even reaching drinking water. The presence of many of these compounds in surface and drinking water has been reported in screening studies, but there is still a lack of analytical methods capable of quantifying them. Herein, we propose a method combining mixed-mode-solid-phase extraction (MM-SPE) as pre-concentration technique and mixed-mode liquid chromatography (MMLC) coupled to tandem mass spectrometry as determination technique for the quantitative determination of 23 target PMOCs in surface and drinking water samples. When compared to reversed-phase liquid chromatography, the MMLC protocol has proven to be superior in both retentive capabilities and peak shape for ionic compounds, while performing also well for neutrals. The overall method performance was satisfactory with limits of quantification under 50 ng L⁻¹ for most of analytes in both surface and drinking water. The relative standard deviation was lower than 20% and average recovery was 78 and 80% in surface and drinking water, respectively. The method was applied to 15 water samples collected in Spain, where 17 out of the 23 target PMOCs were quantified in at least one sample. Among them, 6 chemicals (e.g. benzyltrimethylammonium) are reported and/or quantified here for the first time.

Introduction

Concerns about the safety of drinking water sources and the levels of ubiquitous pollution in the water cycle have been growing in recent years. Thus, there is a large amount of published research work to facilitate the authorities the preparation of directives on the restriction of production and discharge of pollutants to the environment ¹. However, many regulatory measures have focused on persistent, bioaccumulative and toxic chemicals (PBT), which, although worrying, are usually not very mobile through the water cycle, since they tend to settle or accumulate in biota and sediments. In contrast, persistent and mobile organic contaminants (PMOCs) and specially, those ones that are also toxic (i.e. persistent, mobile and toxic, PMTs) ² have been much less studied although they present a great ability to spread through the water cycle ³. One of the main reasons for the lack of information about these compounds is the limited existence of analytical methods capable of detecting, and above all, quantifying them, which is considered as an analytical gap for polar contaminants monitoring ⁴.

Although frequently liquid chromatography (LC) based on reversed phase (RP) retention mechanisms has been used to separate organic chemicals in water samples, it is evident that poor behaviour can be expected for the most polar analytes ⁴. Consequently, other alternatives have been developed that perform much better for these compounds, such as hydrophilic interaction liquid chromatography (HILIC) ⁵⁻⁶, supercritical fluid chromatography (SFC) ⁷ or mixed-mode liquid chromatography (MMLC) ⁸.

However, the analytical gap in PMOCs analysis is not only a consequence of the chromatographic separation. The selection of sample preparation methodology is another great challenge since PMOCs have a high affinity for the aqueous medium. Non-discriminant methods based on dewatering such as evaporation ⁹ or freeze-drying ⁸ have been proposed but they are time-consuming and lead to extremely complex extracts and consequently, strong matrix effects when the analysis are performed by LC coupled to mass spectrometry (LC-MS). Solid-phase extraction (SPE) is the most popular extraction technique in the analysis of water samples. The most frequently selected polymers in the analysis of non-polar compounds are those based on RP, including polymeric hydrophilic sorbents as for example OASIS HLB, with some applications towards the determination of polar compounds ¹⁰⁻¹¹. In the case of ionic/basic/acidic analytes, ion exchangers and mixed-mode SPE are more appropriate ¹²⁻¹³.

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3 Due to the lack of both enrichment and determination methods for PMOCs analysis in water
4 samples, only few reliable monitoring data as regards the real levels of these compounds
5 circulating through the water cycle, and even reaching water sources and drinking water are
6 available^{7-8, 13}. Thus, the aim of this study is to propose an analytical methodology that can be
7 used to provide (for the first time in many cases) quantitative data on 23 selected PMOCs
8 occurrence in different water samples based on two newly developed SPE methods and exploiting
9 the promising results we had previously observed by MMLC in high-resolution-MS screening⁸.
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18 **Materials and Methods**

21 **Chemicals and reagents**

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23 HPLC grade acetonitrile (ACN) was purchased from Merck (Darmstadt, Germany). Acetic acid
24 ($\geq 99\%$), formic acid ($\geq 99\%$) and ammonium hydroxide solution (25%) were purchased from
25 Sigma-Aldrich (Steinheim, Germany). The methanolic solution of ammonia (7N) used for
26 preparing the conditioning and elution solutions in SPE was acquired from Acros Organics (Geel,
27 Belgium). 23 PMOCs, with logD values at pH 7 ranging from -3.7 to 3.4 (average -0.8) were
28 investigated. Detailed information regarding these analytes, including physico-chemical
29 properties and suppliers is given in Table S1. Further information regarding the uses and ECHA
30 registry number of these analytes can be found in the Supporting Information (Table S2).
31 Discussion on analyte selection is provided in the Results and Discussion section. A 1 M
32 ammonium acetate aqueous solution at pH 5.5 was obtained by preparing a 1 M acetic acid
33 solution, which was then made to pH 5.5 by adding aqueous ammonium hydroxide until the
34 desired pH. This solution was further diluted as needed for preparing the LC eluents.
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48 **Samples and sample preparation**

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50 The analyzed samples were obtained from different locations in Galicia (NW Spain) including
51 surface (M1 – M9) and drinking water (M10 – M12). Commercial bottled water (M13 – M15) was
52 acquired in a local supermarket. The sampling campaign was programmed during the year 2017.
53 Sampling location is given in the supporting information (Figure S1). Each sample was analyzed
54 three times.
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3 The samples were filtered through 0.45 μm PVDF filters (Merck Millipore, Darmstadt, Germany)
4 and submitted to two different SPE protocols using mixed-mode cartridges, OASIS WAX and
5 OASIS WCX (Waters, Milford, MA, USA), as summarized in Figure 1. The OASIS WAX protocol
6 was as follows: 100 mL of sample were passed through an OASIS WAX 150 mg cartridge
7 previously conditioned with 5 mL of methanol (2% formic acid) and 5 mL of Milli-Q water. Then,
8 the cartridge was dried and eluted with 6 mL of methanol (5 % ammonia). The extract was finally
9 evaporated to dryness and reconstituted with 200 μL of Milli-Q water:ACN (9:1), filtered through
10 a 0.22 μm PP filter (Merck Millipore) and injected in the LC-MS system operating in electrospray
11 (ESI) negative mode. On the other hand, in the OASIS WCX protocol, the sample was passed
12 through a 150 mg cartridge previously conditioned with 5 mL of methanol (5% ammonia) and 5
13 mL of Milli-Q water. After drying, the analytes were eluted using 6 mL of methanol (2% formic
14 acid) and this extract was treated as explained for WAX cartridges, but finally injected in LC-MS
15 operating in the ESI positive mode.
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28 The protocol used for OASIS HLB (Waters) cartridges in the comparison studies was as follows:
29 100 mL of sample were loaded in a cartridge (200mg) conditioned with 5 mL of methanol and 5
30 mL of Milli-Q water. After drying, the analytes were eluted using 10 mL of methanol. These
31 extracts were submitted to the same concentration step than Oasis WAX and Oasis WCX
32 cartridges and injected in both ESI positive and negative modes.
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39 **Determination conditions**

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41 The liquid chromatography-tandem mass spectrometry (LC-MS/MS) system comprised an
42 Acquity UPLC H-class chromatograph coupled to a triple quadrupole mass spectrometer (QQQ)
43 Xevo TQD (Waters Corp., Milford, MA, USA) with an ESI source. Nitrogen, used as desolvation
44 and cone gas, was provided by a nitrogen generator (Peak Scientific, Scotland, UK). Argon, for
45 collision induced dissociation, was purchased from Praxair (Madrid, Spain). Ionization was
46 performed either in positive and negative modes using the following parameters: 3.5 and 1.5 kV
47 (capillary voltage in ESI positive and negative modes, respectively), 150 $^{\circ}\text{C}$ (source temperature),
48 400 $^{\circ}\text{C}$ (desolvation temperature), 650 L/h (desolvation gas- N_2 flow) and 10 L/h (cone gas- N_2
49 flow). Collision energy (CE) and cone voltage (CV) values were adjusted individually for each
50 compound. MS analyses were done in Selected Reaction Monitoring (SRM) mode recording two
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precursor/product ion transitions per compound. Selected ions, together with their corresponding CE and CV values are listed in Table S3.

The MMLC column used was an Acclaim Trinity P1 (2.6 μm particle size; 3 mm internal diameter and 50 mm length), supplied by Thermo (Waltham, MA, USA). The final LC method, (taken from our previous PMOC screening methodology)⁸. Eluent A consisted of water:acetonitrile (98:2, v/v), containing 5 mM ammonium acetate solution (pH 5.5), while eluent B was water-acetonitrile (20:80, v/v) containing 20 mM ammonium acetate solution (pH 5.5). Elution was carried out by a linear increase from 0% solvent A to 100% solvent B in 10 min (held for 15 min), i.e. a dual gradient of both organic modifier and ammonium acetate concentration. The injection volume was 5 μL .

For the chromatographic behavior study, the MMLC column was compared with a typical RPLC column, a SymmetryShield RP18 column (3.5 μm , 2.1x100 mm) from Waters. Two different gradients were considered 1) the same organic modifier gradient than MMLC and 2) a typical RPLC gradient from low (2% ACN) to high (98% ACN) organic modifier in 10 min, with a final isocratic (98% ACN) step of 15 min. In this last case, ammonium acetate (5 mM, prepared by adding equimolar amounts of acetic acid and ammonium hydroxide, pH 7) was added to both aqueous and organic mobile phases.

Method performance evaluation

The analytical parameters evaluated were linearity, limits of detection and quantification (LODs and LOQs), repeatability, recovery and matrix effects. The linearity was evaluated in the concentration range between the LOQ and 500 ng mL^{-1} for each analyte through the injection in triplicate of standards at 7 different concentration levels. The instrumental LODs and LOQs (iLODs and iLOQs) were determined through the injection of the same standards and established as the minimum concentration that provided a signal to noise ratio (S/N) of 3 and 10, respectively. The method LODs and LOQs (mLODs and mLOQs) were calculated by spiking real drinking and river water samples at the 50 and 100 ng L^{-1} level (depending on the ESI mode), submitting them to the entire protocol, checking the S/N ratio and extrapolating these concentrations to a S/N of 3 and 10, respectively. For those compounds present in the procedural blank, 1,3-di-*o*-tolylguanidine (DTG), 1,3-diphenylguanidine (DPG), trifluoromethanesulfonic acid (TFMSA),

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3 naphthalene-1-sulfonic acid (NSA), ϵ -caprolactam (CAP) and tri-(2-chloroisopropyl)phosphate
4 (TCPP), the mLOD and mLOQ were also estimated by multiplying by 3 and 10 the standard
5 deviation of the signal in the procedural blank (n=3), respectively. For these compounds, from the
6 two estimation methods, the one that provided the highest mLODs and mLOQs was selected.
7 Instrumental or full methodology precision was measured by the relative standard deviation (RSD)
8 of 5 consecutive injections of standards (iRSD) or three replicates of a spiked sample (1000 ng
9 L⁻¹ and 50 or 100 ng L⁻¹ level) submitted to the entire protocol (mRSD), respectively. Matrix effects
10 (%ME) were calculated at the 1000 ng L⁻¹ level as: %ME = [(A_{se} - A_{use})/A_{ps}] × 100, where A_{se} is
11 the response measured for the spiked extract of a real sample (drinking or surface water), A_{use} is
12 the response for an un-spiked extract of the same sample and, finally, A_{ps} the response for a pure
13 standard ¹⁴. Therefore, a ME value of 100% correspond to no variations in the efficiency of ESI
14 ionization between real sample extracts and pure standard solutions. The method trueness (n=4)
15 was evaluated in surface and drinking water samples at two different addition levels: 1000 ng L⁻¹
16 and 50 (when working in ESI positive mode) or 100 ng L⁻¹ (ESI negative mode), using the standard
17 addition method over final extracts for quantification.
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35 **Results and discussion**

36 **Selection of analytes**

37 The list of studied compounds was built including polar analytes (logD at pH 7 lower than 3.5) and
38 includes chemicals which are positively (10) or negatively (9) charged or even neutral (4) at
39 natural pH (Table S1). Most of the compounds were taken from prioritization studies of PMOCs
40 carried out within the PROMOTE consortium ¹⁵⁻¹⁶. Thus, from the substances registered under
41 REACH, a thorough selection was made by Schulze et al. on the basis of persistency and mobility
42 into the water cycle, produced or imported tonnage and potential release to the environment ¹⁶,
43 as well as availability of standards. In addition to the substances prioritized from REACH, some
44 other polar analytes were added since they were already identified in water samples in our
45 previous screening studies (e.g. adamantan-1-amine - AMANT) ^{8-9, 17}. Thus, 23 compounds were
46 taken from the highly ranked PMOCs list and from PROMOTE project findings ¹⁶⁻¹⁷. Among them,
47 there are some already known PMOCs, such as TCPP or acesulfame (ACE), whose presence in
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3 the environment has been frequently reported ¹⁸⁻¹⁹, but also other PMOCs whose occurrence data
4 had not previously been reported or very scarce literature existed, such as DTG and TFMSA ⁸⁻⁹.
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9 **Comparison of MMLC and RPLC**

10 Two chromatographic approaches were compared: MMLC using an Acclaim Trinity column and
11 RPLC using a SymmetryShield RP18 column and the conditions described in the Materials and
12 Methods section. The MMLC column provides three different retention mechanism since it
13 consists of nano-polymer/silica hybrid particles with the inner-pore area modified with an organic
14 layer that provides both RP and weak anion-exchange properties whereas the outer-pore area is
15 modified with strong cation-exchange functionality.
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23 Figure 2 shows a comparison in terms of retention factors (k) and peak shape, expressed as full
24 width at half maximum (FWHM), for the three studied separations. The MMLC column presented
25 the highest retention factors (average 7.2 ± 2.5) and lowest FWHM (average 13.3 ± 3.6 s),
26 compared to retention factors of (5.1 ± 2.6) and (4.9 ± 2.8), and FWHM of (21.3 ± 12.5) and (20.9
27 ± 13.5) s, in the case of both RPLC separations. The differences for both parameters on the three
28 chromatographic conditions were statistically significant for a one-way ANOVA test ($p=0.008$ and
29 0.022 , respectively). Moreover, in both RPLC experiments only 21 analytes could be included in
30 the Box-Whisker plots (Figure 2) and calculation of average, since peaks for methyl sulfate (MS)
31 and 1,4-diazabicyclo-[2.2.2] octane (DABCO) were not present in the chromatograms. For most
32 of the neutral compounds, i.e. methylbenzenesulfonamide (MBSA), dapsone (DAP), bisphenol S
33 (BPS) and TCPP, the peak shape and retention factors are similar using both columns (Figures
34 S2 and S3), with the only exception of CAP, that presented good peak shape with both columns,
35 but the retention factor was higher using RPLC. For the positively charged compounds, e.g. 1-
36 ethyl-3-methylimidazolium (EMIM) and N-(3-(dimethylamino)-propyl)methacrylamide (MAPMA),
37 the retention factors were comparable (Figure S3), however in terms of peak shape, the MMLC
38 column performed much better (Figure S2). Within this group, the less polar analytes (with $\log D$
39 at $\text{pH } 7 > 1$) DTG and DPG presented a similar peak shape and retention factor with both columns.
40 All the negatively charged compounds (e.g. TFMSA and ACE) were poorly retained using the
41 RPLC column (Figures S2 and S3). Thus, the use of MMLC provided good retention and peak
42 shape for all the considered compounds.
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3 The instrumental method using the MMLC column was validated in terms of linearity, repeatability
4 and LODs and LOQs, as described in the method performance evaluation section (results
5 compiled in Table S4). Acceptable linearity, with R^2 (LOQ – 500 ng mL⁻¹) higher than 0.992, and
6 precision, with iRSD (50 ng mL⁻¹) lower than 8%, were obtained. iLODs ranged from 8 to 2344 ng
7 L⁻¹ (median 155 ng L⁻¹).
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15 **Sample preparation**

16 Two MM-SPE sorbents (OASIS WAX and OASIS WCX) and a hydrophilic RP sorbent (OASIS
17 HLB) were evaluated following the protocols described in Materials and Methods section and
18 Figure 1. With OASIS WAX cartridges, analytes are retained by weak anionic exchange (WAX)
19 through secondary amine groups and RP, while OASIS WCX sorbent retains the analytes by
20 weak cationic exchange (WCX) through carboxylic acid groups and RP. Based on the charge
21 state, chemical structure and retention mode, the OASIS WAX extracts were analyzed for the ESI
22 (-) ionizable compounds, OASIS WCX extracts for the ESI (+) ionizable compounds and the
23 OASIS HLB for all the analytes. Figure 3 shows a comparison in terms of apparent recovery
24 between the protocols with OASIS WAX or WCX (back columns) and the pure RP protocol with
25 OASIS HLB (front columns). For analytes with neutral charge at the working pH, such as MBSA,
26 the apparent recovery is similar or even better when using HLB cartridges, except for BPS which
27 is partially negatively charged at the natural pH of water and showed a better performance using
28 WAX cartridges. However, for both positively or negatively charged compounds the apparent
29 recoveries were higher when the MM-SPE sorbents were used with the only exception of NSA,
30 due to the ionic exchange interactions, which improve retention of charged analytes. Therefore,
31 the two protocols OASIS WCX and OASIS WAX were selected in order to improve the extraction
32 of the more polar PMOCs.
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50 The breakthrough volume was also investigated for both selected MM-SPE protocols. Extractions
51 were carried out with two cartridges connected in series and sample volumes of 50, 100, 250 and
52 500 mL of ultrapure and surface water spiked with 10 ng mL⁻¹ were percolated through the two
53 cartridges. After disassembling, both cartridges were eluted independently with the appropriate
54 solvent (see Materials and Methods). These results are shown in the supporting information
55 (Table S5). Analytes were mainly found in the upper cartridges, only negligible amounts, lower
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3 than 8%, were found in the second cartridge for the most polar compounds (e.g. CAP) when
4 percolating 500 mL of water and percentages lower than 1% (for 4 analytes only) were found
5 when analyzing 100 mL of water. So, no significant breakthrough was observed for sample
6 volumes up to 0.5 L and concentration factors up to 2500 can be reached if necessary. However,
7 since mLODs were deemed satisfactory (see Method Validation section), 100 mL were selected
8 as final sample volume due to practical reasons related to sampling and storage facilities.
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15 The introduction of a washing step is commonly applied when MM cartridges are used to eliminate
16 matrix interferences and reduce matrix effects.²⁰ Specifically, for OASIS WAX and WCX, MeOH
17 is the washing solvent as it disrupts reversed-phase interactions, while analytes remaining retained
18 by ion-exchange and afterwards eluted with methanol containing an acid or salt. Thus, ultrapure
19 water samples spiked with 10 ng mL⁻¹ were submitted to the entire protocols where a washing
20 step using 6 mL of MeOH was introduced before the final elution step using methanol containing 5
21 % ammonia or methanol containing 2% formic acid, for OASIS WAX or WCX, respectively. These
22 washing fractions were concentrated and analyzed following the same protocol than the final
23 extracts. The results of these tests are shown in Figure S4. whereas it can be observed the most
24 acidic/basic (or ionic) analytes do not elute in this washing step. However, all the neutral analytes,
25 such as BPS, DAP or TCPP are completely eluted in these washing fractions. Also, some ionic
26 species, such as AMANT or AMPSA, were partially eluted in the washes. Thus, the use of such
27 a washing step could not be implemented.
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42 **Evaluation of matrix effects**

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44 Matrix effects were evaluated with two different matrices (surface and drinking water) at 1000 ng
45 L⁻¹ addition level. The obtained results are shown in Figure S5. These values were extremely
46 variable, ranging from a strong signal suppression (for saccharine, SAC) to a signal enhancement
47 (for BPS). For most of the compounds similar matrix effects were observed in both matrices,
48 except for SAC, xylenesulfonic acid (XSA) which show higher matrix effect in surface water and
49 BPS which showed signal enhancement in surface water. Thus, we decided to perform the
50 quantification of the samples by the standard addition method, building the calibration curve over
51 the final extracts for each sample. This protocol is commonly used when external calibration is
52 not suitable for quantification purposes²¹ and no internal standards are available.
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Method validation

The figures of merit for trueness (R%), precision (mRSD), at two concentration levels, and mLOD and mLOQ are shown in Table 1. At the lower spiked level, recoveries and mRSD for some compounds could not be estimated due to different reasons, viz. the presence at higher levels in the samples (i.e. ACE) or higher mLOQ because a) instrumental sensitivity and/or high matrix effect (ABSA, SAC, DABCO and DAP) or b) the levels found in procedural blanks (TCPP and CAP). The trueness of the entire protocol, using the standard addition over the extract methodology, was acceptable (with recoveries higher than 60% except for CAP). The average recovery for surface and drinking water was 79 and 80% at the highest addition level (1000 ng L⁻¹) and 85 and 79 for the lowest addition level (50 ng L⁻¹ in ESI(+) and 100 ng L⁻¹ in ESI(-)). The mRSD values for four replicates varied between 2 and 20 % in surface water and between 2 and 18 % in drinking water. The highest mLODs were found for ABSA in both matrices (100 and 79 ng L⁻¹ in surface and drinking water, respectively). For the other compounds the mLOD varied between 0.02 and 16 ng L⁻¹ and between 0.2 and 21 ng L⁻¹ in surface and drinking water, respectively. These results are in line with that published for that well-known compounds with available literature, such as ACE²², BPS²³, with the exception of TCPP²⁴, while for many other compounds this is the first developed quantitative analytical method.

Occurrence in real samples

The developed method was used to analyze water samples collected during 2017 in different geographical points located in Galicia, NW Spain (Figure S1). The obtained results, compiled in Table 2 show that out of the 23 analytes, 1 (XSA) was quantified in all the analyzed samples except the mineral bottled waters (M13 – M15). Five analytes were found in more than 6 samples and 11 compounds were found in at least one of the analyzed samples. Thus, a total of 17, of the original list of 23 compounds, could be quantified (Table 2). None of the studied compounds was detected in the analyzed bottled water samples (M13 – M15) at levels higher than the mLOD, thus, these samples are not included in Table 2. As an example, Figure 4 shows the MRM chromatograms for all the compounds found in sample M1, which corresponds to a river near a urban settlement and was found to be the most polluted one (16 out of 23 analytes were found in this sample).

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3 Among the quantified compounds there are two common sweeteners, SAC and ACE, which were
4 actually the two analytes detected at higher concentrations (7 and 122 $\mu\text{g L}^{-1}$, respectively, in
5 sample M1). Their levels are, in general, in agreement with those found in surface waters from
6 other origins ^{10, 22}, with the exception of ACE in M1, a surface water collected near an urban
7 settlement, being even higher than those reported by other authors in some wastewater effluents
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15 Other frequently reported in the literature water polar pollutants are BPS, TCPP and, to a less
16 extent, CAP, used industrially as additives in coatings, textiles and plastics. The levels of BPS
17 and TCPP found in this work (LOQ-88 and LOQ-385 ng L^{-1}) are in agreement with those reported
18 in other works that considered them ubiquitous in the water environment ^{18, 25}. Although there are
19 few publications, CAP has also been previously reported in surface waters ²⁶ and was found in
20 this work in 11 out of 15 samples, including tap waters in a concentration range between 50 and
21 352 ng L^{-1} . This high frequency of detection should not be surprising since CAP is extensively
22 used, being its production the highest within the studied compounds (higher than one million of
23 tonnes per year in Europe, Table S2) and pinpointing the need for further environmental research.
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32 Four out of 17 quantified compounds were sulfonates, among them, NSA and XSA were
33 previously reported as water pollutants ^{8, 27-28} whereas 2-acrylamino-2-methylpropane sulfonate
34 (AMPSA) is reported here for the first time. This compound is used in industry for polymerization
35 processes and produced in amounts higher than ten thousand tones per year ²⁹. Regarding
36 trifluoromethanesulfonic acid (TFMSA), its presence was reported (but not quantified) by Zahn et
37 al. ⁹ and in our previous screening study ⁸. With the herein developed method we could quantify
38 this chemical at nearly 1 $\mu\text{g L}^{-1}$ in sample M2 which corresponds to a river water connected to a
39 landfill leachate.
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48 In our previous screening study we also reported DTG as water pollutant for the first time with a
49 detection frequency of 24% in European water samples from different origins ^{8, 17}. In this work,
50 we could now detect and quantify DTG in the two most polluted samples (M1 and M2). This
51 compound is extensively used as process regulator and reagent in vulcanization. The same
52 industrial use is given to its analog DPG, being the annual production of the latter 10 times higher
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To our knowledge, there is no literature reporting the presence of benzyltrimethylammonium (BETMA) and benzyldimethylamine (BDMA) in water samples, both being produced in amounts higher than 100 tonnes per year and used in polymerization processes. In fact, BETMA is used in the manufacturing of ion exchange polymers for removal of charged micropollutants from water (Table S2). Both PMOCs were found in some of the analyzed samples in concentrations between 2.7 and 107 ng L⁻¹.

Finally, AMANT which is a pharmaceutical and MBSA, were found in four and three samples, respectively. Both have been previously reported as water pollutants in a couple of publications³⁰⁻³¹. The levels of MBSA (up to 1.9 µg L⁻¹) found in our study are high enough to be taken into consideration in future studies, since data on occurrence and fate of this chemical is still very scarce.

Conclusions

In this study we developed a method for the quantification of 23 target PMOCs in water samples. By combining MM-SPE and MMLC, the method is suitable to determine such very polar chemicals. 17 out of 23 target compounds were quantified in at least one of the samples analyzed, several of them quantified for the first time. In summary, this work highlights the role of new analytical approaches, which are needed to provide environmental data on very polar analytes. MMLC can be used for that, since it improves the determinability of polar ionic (basic/acidic) analytes while permitting the determination of neutral chemicals.

Acknowledgements

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Supporting information available

Table S1: Selected PMOCs physico-chemical and supplier information. Table S2. Selected PMOCs application information from REACH dossiers (as to December 2018). Table S3: Mass spectrometric detection parameters: cone voltage (CV), selected m/z precursor (Q1 mass) and product ion (Q3 mass) and collision energy (CE) for the two transitions used in every case. Table S4: Retention time (tr), instrumental limits of detection, and quantification (iLOD and iLOQ), repeatability (iRSD) and linearity (R2). Table S5: Breakthrough volume evaluation. Percentage of

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3 analyte found in second cartridge (2 cartridges connected in series). Figure S1: Location of
4 samples collected in Galicia (NW Spain) during May-June, 2017 (map source: NASA,
5 <https://visibleearth.nasa.gov/view.php?id=55802>; File: Spain.A2001148.1125.250m.jpg; Credit:
6 Jacques Descloitres, MODIS Land Rapid Response Team at NASA/GSFC).. Figure S2:
7 Chromatograms obtained by (1) MMLC using the final gradient shown in the materials and
8 methods section, (2) RPLC using the same conditions than MMLC and (3) RPLC using a typical
9 gradient from low (2% ACN) to high (98% ACN) organic modifier with AcONH₄ set at 5 mM (pH
10 7) in both eluents. Figure S3. Retention factors for each individual analyte in the three
11 chromatographic separations, MMLC, RP gradient 1 (using the same conditions than MMLC) and
12 RPLC gradient 2 (using a typical gradient from low (2% ACN) to high (98% ACN) organic modifier
13 with AcONH₄ set at 5 mM (pH 7) in both eluents.. Figure S4: Evaluation of the washing step in
14 SPE protocol. Percentage of analyte found in a washing fraction (6 mL of MeOH) introduced
15 before the final elution (n=3). Figure S5: Matrix effects (% ME) of the selected compounds in
16 surface and drinking water (1000 ng L⁻¹ addition level).
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20 References

- 21 (1). Commission, E. Directive 2000/60/EC of the European Parliament and of the Council of 23
22 October 2000 establishing a framework for Community action in the field of water policy.
23 (2). Neumann, M. Protecting the sources of our drinking water – A revised proposal for
24 implementing criteria and an assessment procedure to identify Persistent, Mobile and Toxic
25 (PMT) and very Persistent, very Mobile (vPvM) substances registered under REACH.
26 *Environmental Agency* **2017**, ISSN 2363-8273.
27 (3). Reemtsma, T.; Weiss, S.; Mueller, J.; Petrovic, M.; González, S.; Barcelo, D.; Ventura, F.;
28 Knepper, T. P. Polar Pollutants Entry into the Water Cycle by Municipal Wastewater: A European
29 Perspective. *Environ. Sci. Technol.* **2006**, *40* (17), 5451-5458.
30 (4). Reemtsma, T.; Berger, U.; Arp, H. P. H.; Gallard, H.; Knepper, T. P.; Neumann, M.; Quintana,
31 J. B.; Voogt, P. d. Mind the Gap: Persistent and Mobile Organic Compounds—Water
32 Contaminants That Slip Through. *Environ. Sci. Technol.* **2016**, *50* (19), 10308-10315.
33 (5). Buszewski, B.; Noga, S. Hydrophilic interaction liquid chromatography (HILIC)—a powerful
34 separation technique. *Anal. Bioanal. Chem.* **2012**, *402* (1), 231-247.
35 (6). Nováková, L.; Havlíková, L.; Vlčková, H. Hydrophilic interaction chromatography of polar and
36 ionizable compounds by UHPLC. *TrAC Trends Analyt. Chem.* **2014**, *63*, 55-64.
37 (7). Bieber, S.; Greco, G.; Grosse, S.; Letzel, T. RPLC-HILIC and SFC with Mass Spectrometry:
38 Polarity-Extended Organic Molecule Screening in Environmental (Water) Samples. *Anal. Chem.*
39 **2017**, *89* (15), 7907-7914.
40 (8). Montes, R.; Aguirre, J.; Vidal, X.; Rodil, R.; Cela, R.; Quintana, J. B. Screening for Polar
41 Chemicals in Water by Trifunctional Mixed-Mode Liquid Chromatography–High Resolution Mass
42 Spectrometry. *Environ. Sci. Technol.* **2017**, *51* (11), 6250-6259.
43 (9). Zahn, D.; Frömel, T.; Knepper, T. P. Halogenated methanesulfonic acids: A new class of
44 organic micropollutants in the water cycle. *Water Res.* **2016**, *101*, 292-299.
45 (10). Arbeláez, P.; Borrull, F.; Pocurull, E.; Marcé, R. M. Determination of high-intensity
46 sweeteners in river water and wastewater by solid-phase extraction and liquid chromatography–
47 tandem mass spectrometry. *J. Chromatogr. A* **2015**, *1393*, 106-114.
48 (11). Nosek, K.; Styszko, K.; Golas, J. Combined method of solid-phase extraction and GC-MS
49 for determination of acidic, neutral, and basic emerging contaminants in wastewater (Poland). *Int.*
50 *J. Environ. Anal. Chem.* **2014**, *94* (10), 961-974.
51 (12). Gago-Ferrero, P.; Schymanski, E. L.; Bletsou, A. A.; Aalizadeh, R.; Hollender, J.; Thomaidis,
52 N. S. Extended Suspect and Non-Target Strategies to Characterize Emerging Polar Organic
53 Contaminants in Raw Wastewater with LC-HRMS/MS. *Environ. Sci. Technol.* **2015**, *49* (20),
54 12333-12341.
55 (13). Köke, N.; Zahn, D.; Knepper, T. P.; Frömel, T. Multi-layer solid-phase extraction and
56 evaporation—enrichment methods for polar organic chemicals from aqueous matrices. *Anal.*
57 *Bioanal. Chem.* **2018**, *410* (9), 2403-2411.
58 (14). Matuszewski, B. K.; Constanzer, M. L.; Chavez-Eng, C. M. Strategies for the Assessment
59 of Matrix Effect in Quantitative Bioanalytical Methods Based on HPLC–MS/MS. *Anal. Chem.*
60 **2003**, *75* (13), 3019-3030.

- 1
2
3 (15). Arp, H. P. H.; Brown, T. N.; Berger, U.; Hale, S. E. Ranking REACH registered neutral,
4 ionizable and ionic organic chemicals based on their aquatic persistency and mobility. *Environ.*
5 *Sci.: Processes Impacts* **2017**, *19* (7), 939-955.
- 6 (16). Schulze, S.; Sättler, D.; Neumann, M.; Arp, H. P. H.; Reemtsma, T.; Berger, U. Using
7 REACH registration data to rank the environmental emission potential of persistent and mobile
8 organic chemicals. *Sci. Total Environ.* **2018**, *625*, 1122-1128.
- 9 (17). PROMOTE Project website <http://www.ufz.de/promote/index.php?en=33638> Last accessed
10 09.02.2019.
- 11 (18). van der Veen, I.; de Boer, J. Phosphorus flame retardants: Properties, production,
12 environmental occurrence, toxicity and analysis. *Chemosphere* **2012**, *88* (10), 1119-1153.
- 13 (19). Wode, F.; van Baar, P.; Dünnbier, U.; Hecht, F.; Taute, T.; Jekel, M.; Reemtsma, T. Search
14 for over 2000 current and legacy micropollutants on a wastewater infiltration site with a UPLC-
15 high resolution MS target screening method. *Water Res.* **2015**, *69*, 274-283.
- 16 (20). Senta, I.; Krizman, I.; Ahel, M.; Terzic, S. Integrated procedure for multiresidue analysis of
17 dissolved and particulate drugs in municipal wastewater by liquid chromatography–tandem mass
18 spectrometry. **2013**, *405* (10), 3255-3268.
- 19 (21). Stüber, M.; Reemtsma, T. Evaluation of three calibration methods to compensate matrix
20 effects in environmental analysis with LC-ESI-MS. *Anal. Bioanal. Chem.* **2004**, *378* (4), 910-916.
- 21 (22). Hermes, N.; Jewell, K. S.; Wick, A.; Ternes, T. A. Quantification of more than 150
22 micropollutants including transformation products in aqueous samples by liquid chromatography-
23 tandem mass spectrometry using scheduled multiple reaction monitoring. *J. Chromatogr. A* **2018**,
24 *1531*, 64-73.
- 25 (23). Yamazaki, E.; Yamashita, N.; Taniyasu, S.; Lam, J.; Lam, P. K. S.; Moon, H.-B.; Jeong, Y.;
26 Kannan, P.; Achyuthan, H.; Munuswamy, N.; Kannan, K. Bisphenol A and other bisphenol
27 analogues including BPS and BPF in surface water samples from Japan, China, Korea and India.
28 *Ecotoxicol. Environ. Saf.* **2015**, *122*, 565-572.
- 29 (24). Regnery, J.; Püttmann, W. Occurrence and fate of organophosphorus flame retardants and
30 plasticizers in urban and remote surface waters in Germany. *Water Res.* **2010**, *44* (14), 4097-
31 4104.
- 32 (25). Wu, L.-H.; Zhang, X.-M.; Wang, F.; Gao, C.-J.; Chen, D.; Palumbo, J. R.; Guo, Y.; Zeng, E.
33 Y. Occurrence of bisphenol S in the environment and implications for human exposure: A short
34 review. *Sci. Total Environ.* **2018**, *615*, 87-98.
- 35 (26). Wang, H.; Wang, C.; Wu, W.; Mo, Z.; Wang, Z. Persistent organic pollutants in water and
36 surface sediments of Taihu Lake, China and risk assessment. *Chemosphere* **2003**, *50* (4), 557-
37 562.
- 38 (27). Alonso, M. C.; Barceló, D. Tracing polar benzene- and naphthalenesulfonates in untreated
39 industrial effluents and water treatment works by ion-pair chromatography-fluorescence and
40 electrospray-mass spectrometry. *Anal. Chim. Acta* **1999**, *400* (1), 211-231.
- 41 (28). Betowski, L. D.; Kendall, D. S.; Pace, C. M.; Donnelly, J. R. Characterization of Groundwater
42 Samples from Superfund Sites by Gas Chromatography/Mass Spectrometry and Liquid
43 Chromatography/Mass Spectrometry. *Environ. Sci. Technol.* **1996**, *30* (12), 3558-3564.
- 44 (29). ECHA (2018) [https://www.echa.europa.eu/information-on-chemicals/registered-](https://www.echa.europa.eu/information-on-chemicals/registered-substances)
45 [substances](https://www.echa.europa.eu/information-on-chemicals/registered-substances). Last accessed 31.07.2018.
- 46 (30). Möhle, E.; Metzger, J. W., Drugs in Municipal Sewage Effluents: Screening and
47 Biodegradation Studies. In *Pharmaceuticals and Care Products in the Environment*, American
48 Chemical Society: 2001; Vol. 791, pp 192-205.
- 49 (31). Richter, D.; Dünnbier, U.; Massmann, G.; Pekdeger, A. Quantitative determination of three
50 sulfonamides in environmental water samples using liquid chromatography coupled to
51 electrospray tandem mass spectrometry. *J. Chromatogr. A* **2007**, *1157* (1), 115-121.
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5 Figure 1: Scheme of the two final analytical protocols.
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7 Figure 2: Retention factors (k) and peak width as Full Width at Half Maximum (s) for the three
8 studied separations: MMLC, RP gradient 1 (using the same conditions than MMLC) and RPLC
9 gradient 2 (using a typical gradient from low (2% ACN) to high (98% ACN) organic modifier and
10 both eluents containing 5 mM AcONH₄, pH 7). Average values \pm SD are represented in brackets.
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13 Figure 3: Apparent recovery for OASIS HLB protocol (front columns) and OASIS WAX or OASIS
14 WCX (back columns).
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16 Figure 4: MRM chromatograms of the compounds found in sample M1.
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Table 1: Figures of merit for the complete protocol in surface and drinking water (n=4).

Compound	Surface water				Drinking water			
	Accuracy, %R (mRSD)		mLOD	mLOQ	Accuracy, %R (mRSD)		mLOD	mLOQ
	1000 ng L ⁻¹	50/100 ng L ⁻¹ (1)	ng L ⁻¹	ng L ⁻¹	1000 ng L ⁻¹	50/100 ng L ⁻¹ (1)	ng L ⁻¹	ng L ⁻¹
TFMSA	92 (10)	95 (6)	0.02	0.1	84 (6)	87 (11)	0.05	0.2
AMPSA	74 (12)	77 (18)	8	28	84 (6)	67 (5)	7	23
MS	70 (11)	73 (20)	3.0	10	67 (14)	67 (15)	0.5	1.7
ABSA	85 (18)	(a)	100	333	80 (18)	(a)	79	263
SAC	65 (20)	(a)	18	60	90 (10)	76 (17)	10	33
SA	60 (5)	75 (6)	6.2	21	81 (11)	71 (16)	11	36
XSA	92 (9)	86 (17)	6.3	21	98 (6)	81 (16)	1.2	3.9
NSA	96 (9)	96 (18)	1.8	6.1	87 (7)	94 (10)	0.4	1.2
ACE	75 (16)	(b)	1.2	4.2	69 (8)	(b)	0.4	1.5
MAPTA	73 (2)	88 (12)	7.3	24	90 (3)	89 (15)	11	36
BETMA	100 (12)	100 (11)	0.1	0.5	87 (11)	78 (14)	0.4	1.3
EMIM	107 (6)	108 (16)	1.5	4.9	99 (2)	86 (11)	0.3	1.1
DTG	86 (6)	83 (14)	0.6	2.1	84 (1)	86 (4)	0.1	0.2
BDMA	80 (14)	80 (15)	0.1	0.3	69 (3)	67 (8)	1.2	4.0
DABCO	64 (17)	(a)	16	53	73 (3)	(a)	21	70
AMANT	90 (11)	82 (5)	0.3	1.1	79 (7)	69 (8)	0.3	0.9
DPG	84 (14)	91 (16)	0.1	0.2	79 (12)	87 (16)	0.1	0.3
MAPMA	77 (13)	82 (14)	0.6	2.0	74 (14)	88 (16)	1.4	4.7
MBSA	80 (5)	76 (20)	16	35	87 (12)	81 (14)	7.9	26
DAP	69 (19)	(a)	15	50	75 (6)	(a)	12	41
BPS	69 (6)	67 (17)	0.1	0.4	80 (10)	83 (16)	0.3	1.1
CAP	56 (19)	(a)	13	43	69 (9)	(a)	13	43
TCPP	62 (3)	(a)	14	46	63 (5)	(a)	14	46

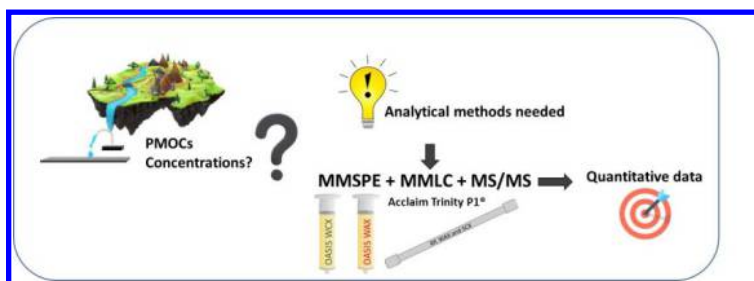
(1) 50 ng L⁻¹ for compounds analyzed in ESI (+) mode and 100 ng L⁻¹ for compounds analyzed in ESI (-) mode. (a) not evaluated, addition level close to LOQs. (b) not evaluated, high concentration in the unspiked sample.

Table 2: Levels of PMOCs found in real samples (concentration \pm SD, ng L⁻¹) (n=3). Sample information is given in the supporting information (Figure S1). M13-15 not shown, since none of the analytes was detected.

Compound	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
TFMSA	n.d.	965 \pm 106	n.d.	n.d.	n.d.	4.2 \pm 0.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AMPMSA	467 \pm 74	n.d.	n.d.	n.d.	n.d.	n.d.	66 \pm 10	38 \pm 2	36 \pm 3	n.d.	n.d.	n.d.
SAC	7707 \pm 1310	618 \pm 74	249 \pm 32	n.d.	77 \pm 12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
XSA	1767 \pm 265	72 \pm 11	357 \pm 46	32 \pm 4	129 \pm 15	65 \pm 9	35 \pm 5	38 \pm 5	26 \pm 1	18 \pm 6	11 \pm 3	31 \pm 8
NSA	331 \pm 59	25 \pm 4	n.d.	n.d.	18 \pm 3	n.d.	79 \pm 10	n.d.	n.d.	n.d.	n.d.	n.d.
ACE	122353 \pm 19576	389 \pm 58	2770 \pm 387	n.d.	553 \pm 88	272 \pm 35	n.d.	n.d.	n.d.	n.d.	n.d.	7.4 \pm 1.5
BETMA	71 \pm 8	59 \pm 9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EMIM	5.6 \pm 0.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DTG	9 \pm 1	2.2 \pm 0.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BDMA	58 \pm 5	107 \pm 11	n.d.	2.7 \pm 0.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DABCO	128 \pm 12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AMANT	48 \pm 7	13 \pm 2	2.7 \pm 0.6	n.d.	1.6 \pm 0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DPG	173 \pm 21	32 \pm 5	3.6 \pm 0.5	n.d.	6.8 \pm 0.9	5.5 \pm 0.8	n.d.	n.d.	n.d.	4.2 \pm 0.6	7.3 \pm 2.1	n.d.
MBSA	982 \pm 137	1911 \pm 248	115 \pm 13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BPS	88 \pm 12	85 \pm 13	2.4 \pm 0.3	2.6 \pm 0.4	18 \pm 3	8.1 \pm 1.1	n.d.	n.d.	1.0 \pm 0.2	n.d.	n.d.	n.d.
CAP	352 \pm 45	91 \pm 12	n.d.	51 \pm 5	191 \pm 28	114 \pm 15	103 \pm 14	59 \pm 6	70 \pm 7	50 \pm 5	82 \pm 9	150 \pm 16
TCP	385 \pm 65	n.d.	n.d.	n.d.	94 \pm 15	89 \pm 13	n.d.	68 \pm 12	117 \pm 11	n.d.	79 \pm 5	132 \pm 11

n.d. not detected or under the mLOQ

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Fig 1.

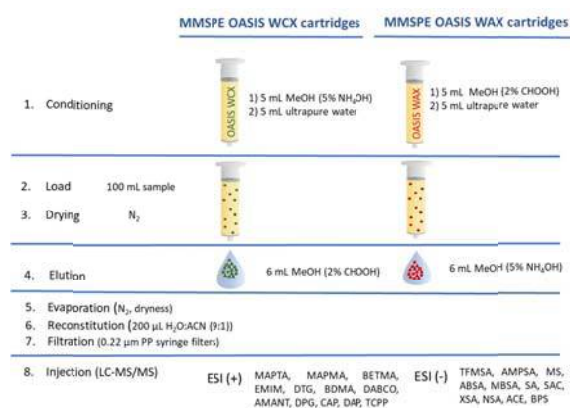


Fig 2

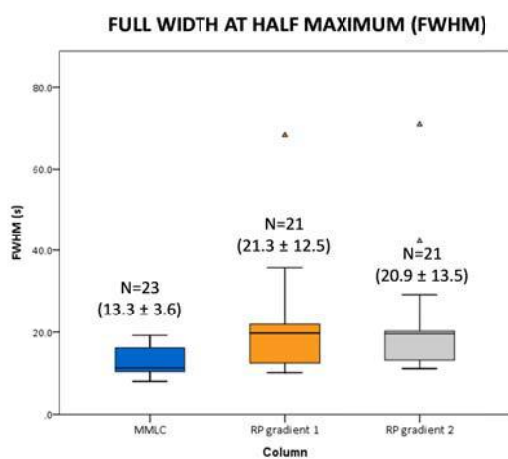
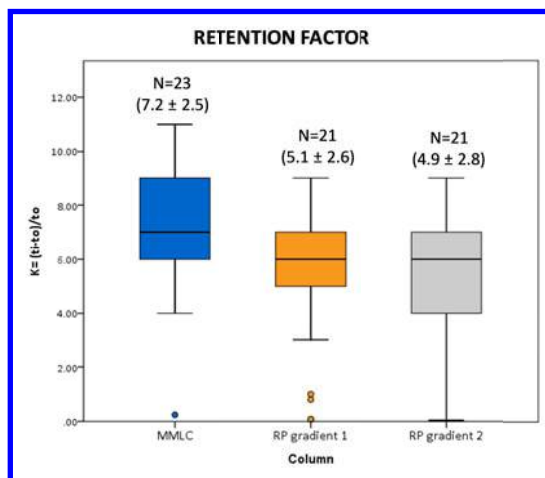


Fig 3

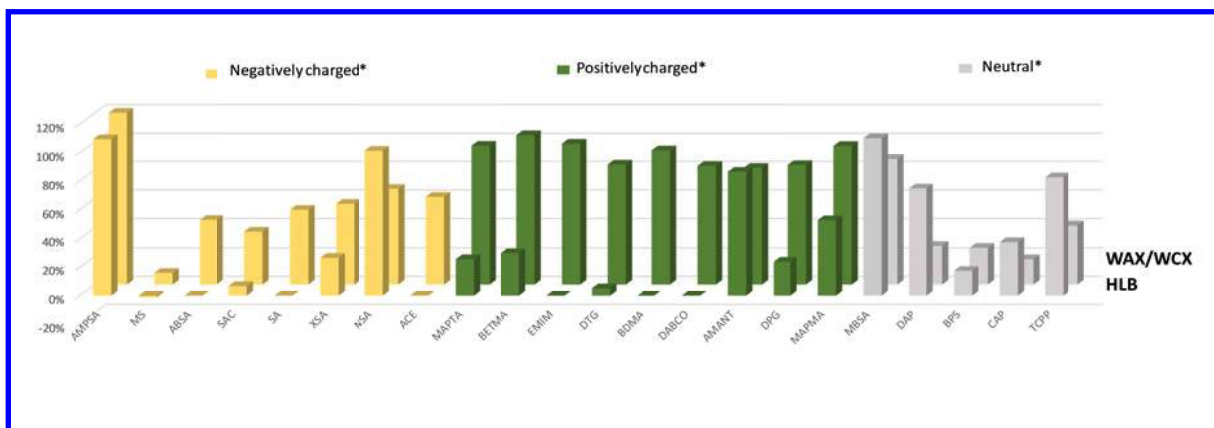
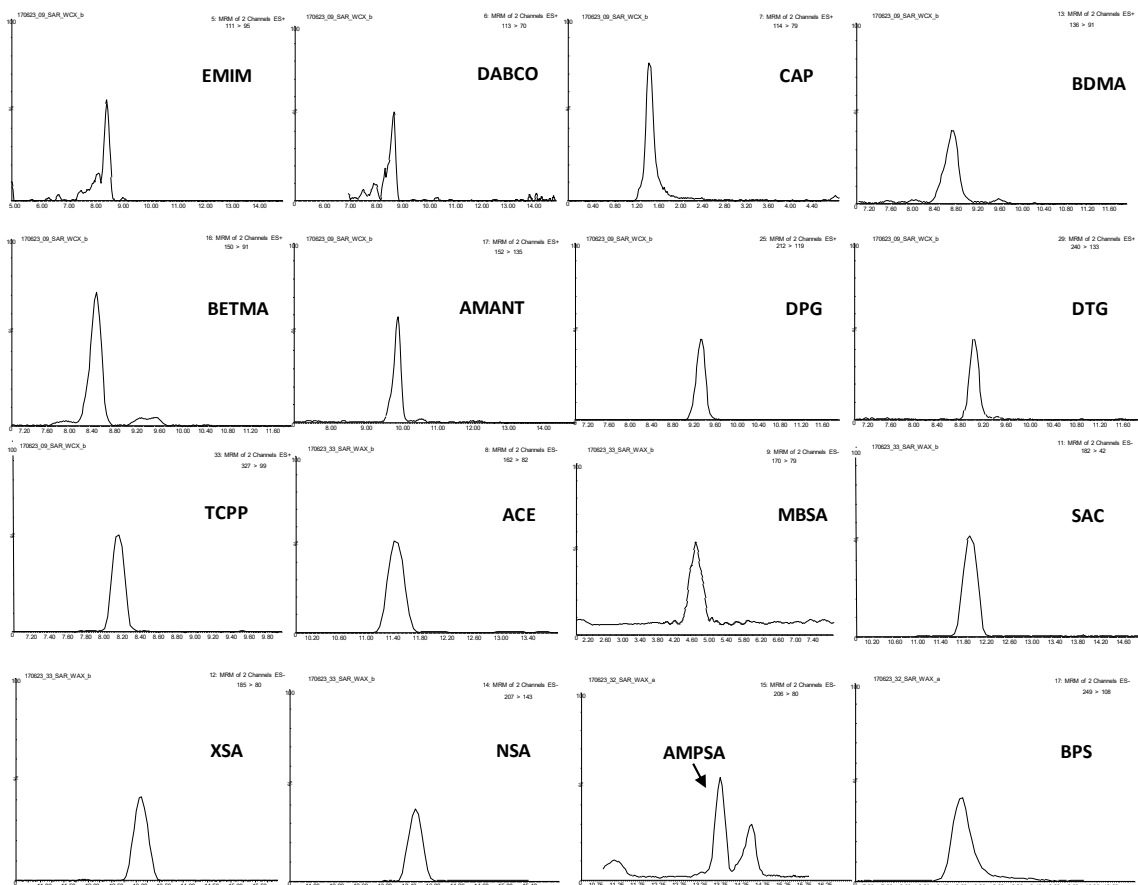


Fig 4



Supporting information to:

Determination of persistent and mobile organic contaminants (PMOCs) in water by mixed-mode liquid chromatography-tandem mass spectrometry.

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Figure S3. Retention factors for each individual analyte in the three chromatographic separations, MMLC, RP gradient 1 (using the same conditions than MMLC) and RPLC gradient 2 (using a typical gradient from low (2% ACN) to high (98% ACN) organic modifier with AcONH_4 set at 5 mM (pH 7) in both eluents).

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Figure S5: Matrix effects (% ME) of the selected compounds in surface and drinking water (1000 ng L^{-1} addition level).

Table S1. Selected PMOCs physico-chemical and supplier information.

Name	Acronym	Charge ⁽¹⁾	LogD (pH 7) ⁽²⁾	pK _a ⁽²⁾	CAS ⁽³⁾	Supplier ⁽³⁾	Purity ⁽³⁾
Trifluoromethanesulfonic acid	TFMSA	-1	-1.23	-3.43	1493-13-6	Sigma Aldrich	≥ 99 %
(Sodium) 2-acrylamino-2-methylpropane sulfonate	AMPASA	-1	-2.71	-0.96	5165-97-9	ABCR GmbH	n.p.
(Sodium) methyl sulfate	MS	-1	-2.84	-2.37	512-42-5	Sigma Aldrich	n.p.
Aniline-3-sulfonic acid	ABSA	-1	-2.04	-3.51	121-47-1	Sigma Aldrich	99%
Saccharine	SAC	-1	-0.49	1.94	81-07-2	Sigma Aldrich	≥ 99%
Aniline-4-sulfonic acid	SA	-1	-2.04	-3.39	121-57-3	Sigma Aldrich	n.p.
Xylenesulfonic acid	XSA	-1	-0.2	-1.94	1300-72-7	Sigma Aldrich	n.p.
Naphthalene-1-sulfonic acid	NSA	-1	-0.23	-1.77	85-47-2	Fluorochem	n.p.
Acesulfame (K)	ACE	-1	-3.06	3.02	55589-62-3	Sigma Aldrich	≥ 99 %
Methacrylamido propyl trimethyl ammonium (chloride)	MAPTA	+1	-3.74	15.9	51410-72-1	ABCR GmbH	50%
Benzyltrimethylammonium (chloride)	BETMA	+1	-2.24	-	56-93-9	Sigma Aldrich	97%
(1-Ethyl-3-methylimidazolium) ethyl sulfate	EMIM	+1	-2.48	-	342573-75-5	Fluka Analytical	≥ 98.5 %
1,3-Di-o-tolylguanidine	DTG	+1	2.25	9.43	97-39-2	Sigma Aldrich	99%
Benzyltrimethylamine	BDMA	+1	0.02	8.9	103-83-3	Serva	r.g.
1,4-diazabicyclo-[2.2.2]octane	DABCO	+1	-2.83	9.76	280-57-9	Sigma Aldrich	≥ 99 %
Adamantan-1-amine	AMANT	+1	-1.49	10.71	768-94-5	Fluorochem	n.p.
1,3-Diphenylguanidine	DPG	+1	1.23	9.38	102-06-7	Sigma Aldrich	97%
N-(3-(dimethylamino)-propyl)methacrylamide	MAPMA	+1	-1.85	9.30	5205-93-6	Fluorochem	n.p.
Methylbenzenesulfonamide	MBSA	0	1.09	10.4	70-55-3	Sigma Aldrich	99 %
Dapsone	DAP	0	1.27	2.39	80-08-0	Sigma Aldrich	99.5%
Bisphenol S	BPS	-0.4 to 0 ⁽⁴⁾	2.17	7.42	80-09-1	Sigma Aldrich	≥ 98 %
ε-Caprolactam	CAP	0	0.31	14.9	105-60-2	Sigma Aldrich	a.s.
Tri-(2-chloroisopropyl)phosphate	T CPP	0	3.36	-	13674-84-5	Sigma Aldrich	a.s.

(1) Charge state of predominant species at working pH range (5.5-7); (2) Calculated with Chemaxon; (3) Information of the commercial standards used in this work. (4) The charge state of BPS is varying in the pH range; n.p. not provided; r.g. reagent grade; a.s. analytical standard

Table S2. Selected PMOCs application information from REACH dossiers (as to December 2018)

Acronym	ECHA n°	Production ⁽¹⁾	Uses ⁽²⁾
TFMSA	1493-13-6	100 - 1 000	Manufacture of chemicals and electrical, electronic and optical equipment
AMPSA	225-948-4	10 000 - 100 000	Intermediate in manufacture of polymers, thermoplastics.
MS	208-142-7	1-10	Surface active agent, laboratory chemical
ABSA	204-473-6	1-10	Intermediate in manufacture of chemicals
SAC	201-321-0	100 - 1 000	Food additive, pharmaceutical and personal care products manufacturing
SA	204-482-5	1 000 - 10 000	Manufacture of pH regulators and water treatment products
XSA	215-090-9	1 000 - 10 000	Reagent in vulcanization or polymerization processes
NSA	201-610-1	n.a.	Intermediate in manufacture of chemicals
ACE	259-715-3	1 000 - 10 000	Food additive, intermediate in manufacture of chemicals
MAPTA	257-182-1	100 - 1000	Manufacture of thermoplastics
BETMA	00-300-3	100 - 1000	Manufacture of electrical, electronic and optical equipment. Polymerization processes.
EMIM	460-100-9	100-1000	Additive in fabrics, textiles, wood and long-life material for outdoor use
DTG	202-577-6	100 - 1000	Manufacture of rubber and polymers
BDMA	203-149-1	100 -1000	Manufacture of polymers, adhesives and sealants and coating products.
DABCO	205-999-9	1 000 - 10 000	Manufacture of plastics, adsorbents, machinery, vehicles and furniture
AMANT	212-201-2	n.a.	Intermediate reagent ⁽³⁾ .
DPG	203-002-1	1 000 - 10 000	Manufacture of paints, coatings, flooring, furniture, toys, etc
MAPMA	226-002-3	1 000 - 10 000	Manufacture of thermoplastics, paints and coatings or adhesives
MBSA	200-741-1	10 - 100	Manufacture of textile, leather or fur, wood and wood products, pulp, paper and paper products, etc
DAP	201-248-4	1 000 - 10 000	Manufacture of polymers, rubber, adhesives, sealants
BPS	201-250-5	10 000 - 100 000	Manufacture of coatings, leather, dyes, paper, etc.
CAP	203-313-2	10 ⁶ – 10 ⁷	Manufacture of plastic, textiles, detergents, fragrances and air fresheners, etc
TCPP	237-158-7	1 - 10	Flame retardant. Manufacture of plastics, adsorbents, machinery, vehicles and furniture, etc

(1) Manufactured and/or imported in the European Economic Area (Tonnes per year), (2) Some uses registered by ECHA . (3) Besides the ECHA registry, main use of this compound is pharmaceutical. n.a.: not available in the REACH dossier

Table S3: Mass spectrometric detection parameters: cone voltage (CV), selected m/z precursor (Q1 mass) and product ion (Q3 mass) and collision energy (CE) for the two transitions used in every case.

Acronym	ESI mode	Q1 mass	Q3 mass	Cone voltage (V)	CE (V)
MAPTA	pos.	185	126	20	10
		185	69	20	30
MAPMA	pos.	171	126	40	10
		171	69	40	20
BETMA	pos.	150	91	35	15
		150	65	35	25
EMIM	pos.	111	83	40	15
		111	96	40	25
DTG	pos.	240	133	50	20
		240	108	50	20
BDMA	pos.	136	91	30	15
		136	65	30	30
DABCO	pos.	113	84	70	25
		113	70	70	35
AMANT	pos.	152	135	40	15
		152	93	40	35
DPG	pos.	212	119	45	20
		212	94	45	30
CAP	pos.	114	79	45	15
		114	96	45	15
DAP	pos.	249	156	45	10
		249	92	45	20
TCPP	pos.	327	99	30	20
		327	251	30	10
TFMSA	neg.	149	99	-40	-20
		149	80	-40	-25
AMPSA	neg.	206	80	-50	-30
		206	135	-50	-15
MS	neg.	111	80	-40	-20
		111	96	-40	-20
ABSA	neg.	172	80	-60	-20
		172	108	-60	-15
MBSA	neg.	170	79	-45	-10
		170	62	-45	-30
SA	neg.	172	80	-60	-20
		172	108	-60	-15
SAC	neg.	182	106	-45	-30
		182	62	-45	-30
XSA	neg.	185	80	-50	-30

		185	121	-50	-20
NSA	neg.	207	143	-50	-20
		207	80	-50	-30
ACE	neg.	162	82	-30	-15
		162	40	-30	-30
BPS	neg.	249	108	-50	-20
		249	156	-50	-30

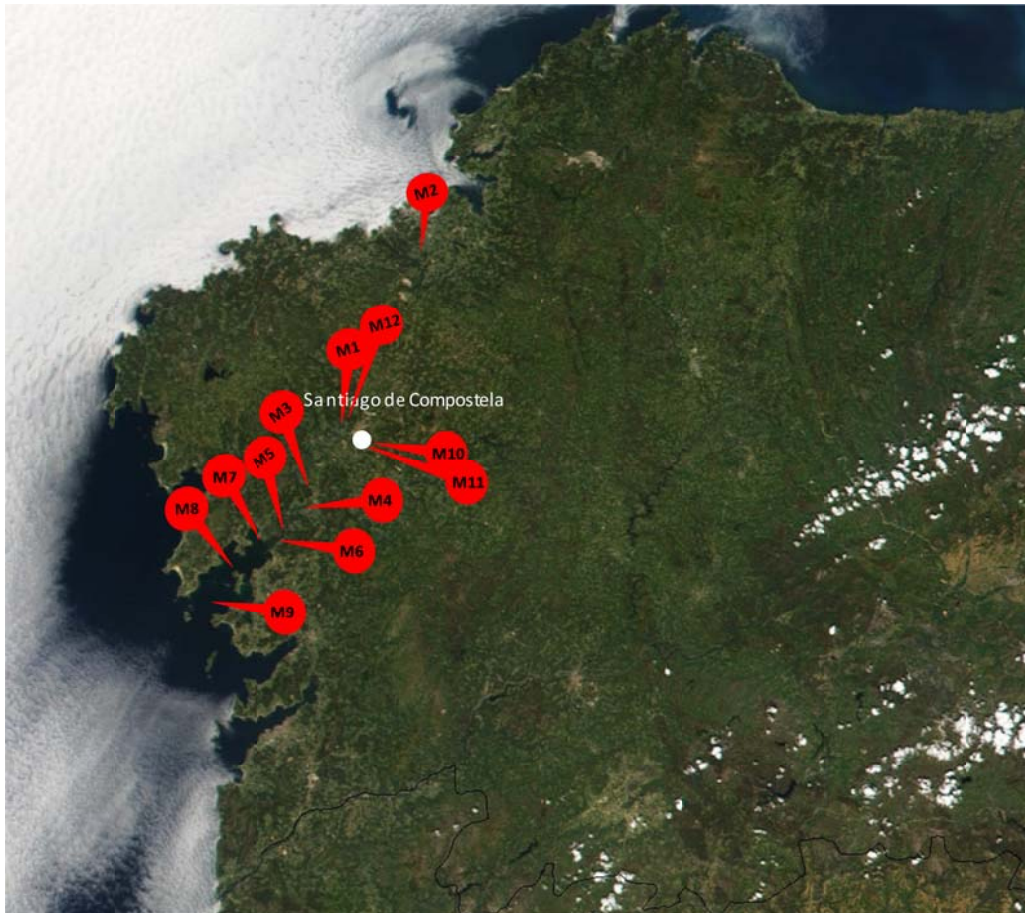
Table S4: Retention time (t_r), instrumental limits of detection, and quantification (iLOD and iLOQ), repeatability (iRSD) and linearity (R^2).

Acronym	t_r (min)	iLOD (ng L ⁻¹)	iLOQ (ng L ⁻¹)	iRSD (n=5) 50ng mL ⁻¹	Linearity (R^2) LOQ-500ng mL ⁻¹
TFMSA	11.4	68	228	3.1	0.999
AMPSA	13.6	223	744	2.6	0.996
MS	9.1	242	806	4.2	0.995
ABSA	9.8	2344	7813	4.0	0.995
SAC	11.6	407	1357	6.3	0.993
SA	10.4	536	1786	5.4	0.994
XSA	13.1	306	1020	5.3	0.998
NSA	13.6	173	576	3.5	0.999
ACE	11.1	145	484	1.8	0.999
MAPTA	6.3	405	1352	5.0	0.995
BETMA	8.6	8	26	2.0	0.998
EMIM	7.5	176	586	3.6	0.998
DTG	9.2	121	404	4.1	0.999
BDMA	9.1	60	201	4.6	0.998
DABCO	8.7	69	231	4.5	0.996
AMANT	10.6	15	51	3.0	0.996
DPG	9.6	32	105	2.7	0.999
MAPMA	6.7	37	122	6.0	0.998
MBSA	5.3	155	516	7.8	0.998
DAP	6.7	22	72	3.8	0.995
BPS	8.2	98	328	3.8	0.996
CAP	1.3	194	647	2.9	0.999
TCPP	7.7	370	1235	4.8	0.992

Table S5: Breakthrough volume evaluation. Percentage of analyte found in second cartridge (2 cartridges connected in series). n.d. not detected

	Analyte in second cartridge (Area %)			
	50 mL	100 mL	250 mL	500 mL
TFMSA	n.d.	n.d.	n.d.	n.d.
AMP	n.d.	n.d.	4.0%	5.4%
MS	n.d.	n.d.	n.d.	1.7%
ABSA	n.d.	n.d.	n.d.	n.d.
SAC	n.d.	n.d.	n.d.	n.d.
SA	n.d.	n.d.	n.d.	n.d.
XSA	n.d.	n.d.	n.d.	n.d.
NSA	n.d.	0.4%	3.9%	5.0%
ACE	n.d.	n.d.	n.d.	3.9%
MAPTA	n.d.	0.2%	0.6%	2.7%
BETMA	n.d.	0.1%	0.3%	3.3%
EMIM	n.d.	n.d.	n.d.	1.4%
DTG	n.d.	n.d.	n.d.	n.d.
BDMA	n.d.	n.d.	n.d.	n.d.
DABCO	n.d.	n.d.	n.d.	4.8%
AMANT	n.d.	n.d.	n.d.	n.d.
DPG	n.d.	n.d.	n.d.	n.d.
MAPMA	n.d.	n.d.	n.d.	n.d.
MBSA	n.d.	n.d.	n.d.	n.d.
DAP	n.d.	n.d.	n.d.	n.d.
BS	n.d.	n.d.	n.d.	n.d.
CAP	n.d.	0.9%	4.6%	7.6%
TCP	n.d.	n.d.	n.d.	n.d.

Figure S1: Location of samples collected in Galicia (NW Spain) during May-June, 2017 (map source: NASA, <https://visibleearth.nasa.gov/view.php?id=55802>; File: Spain.A2001148.1125.250m.jpg; Credit: Jacques Descloitres, MODIS Land Rapid Response Team at NASA/GSFC).



M1: river water near urban settlement.

M2: river water connected to a landfill leachate.

M3 and M4: river water.

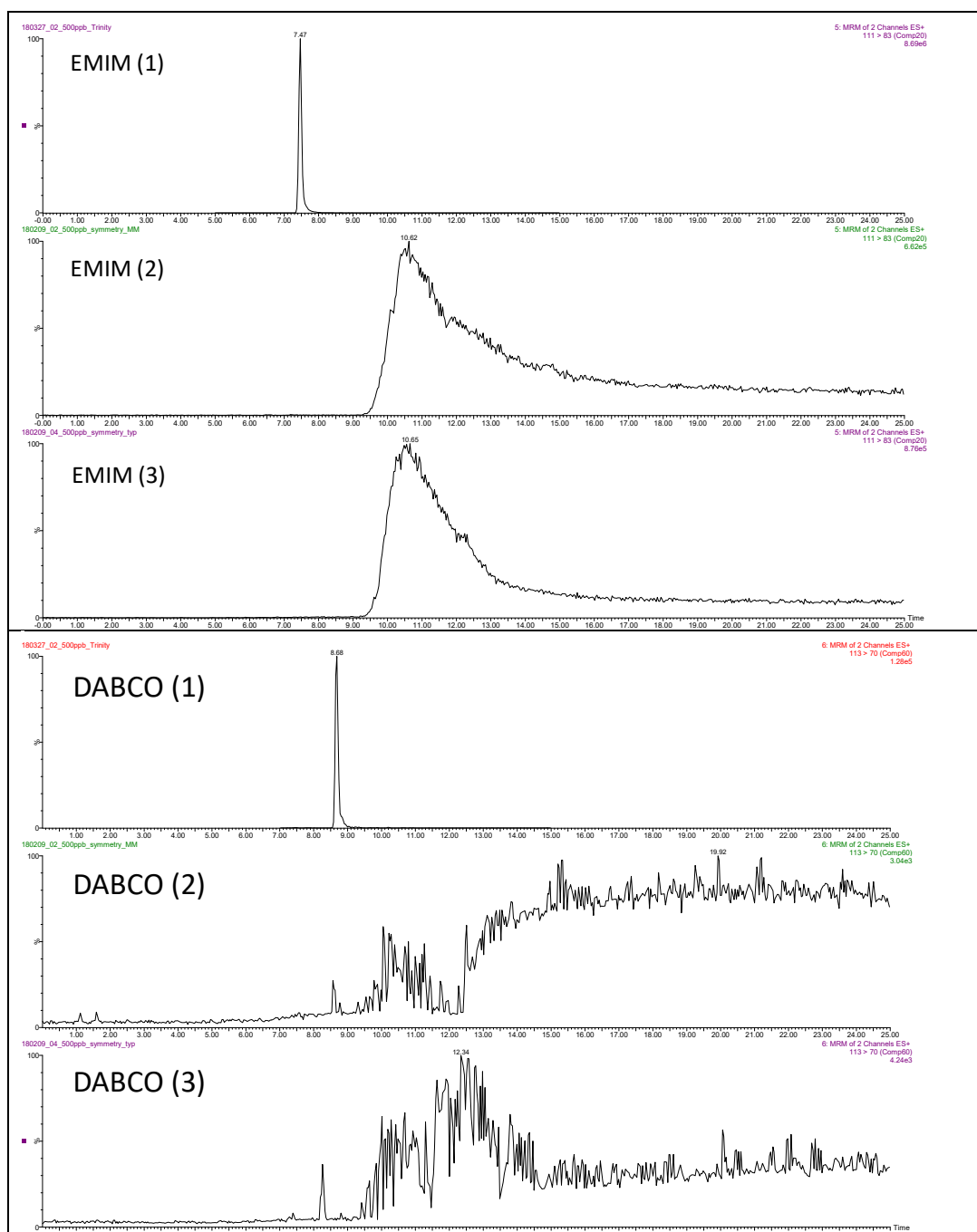
M5 and M6: transitional water.

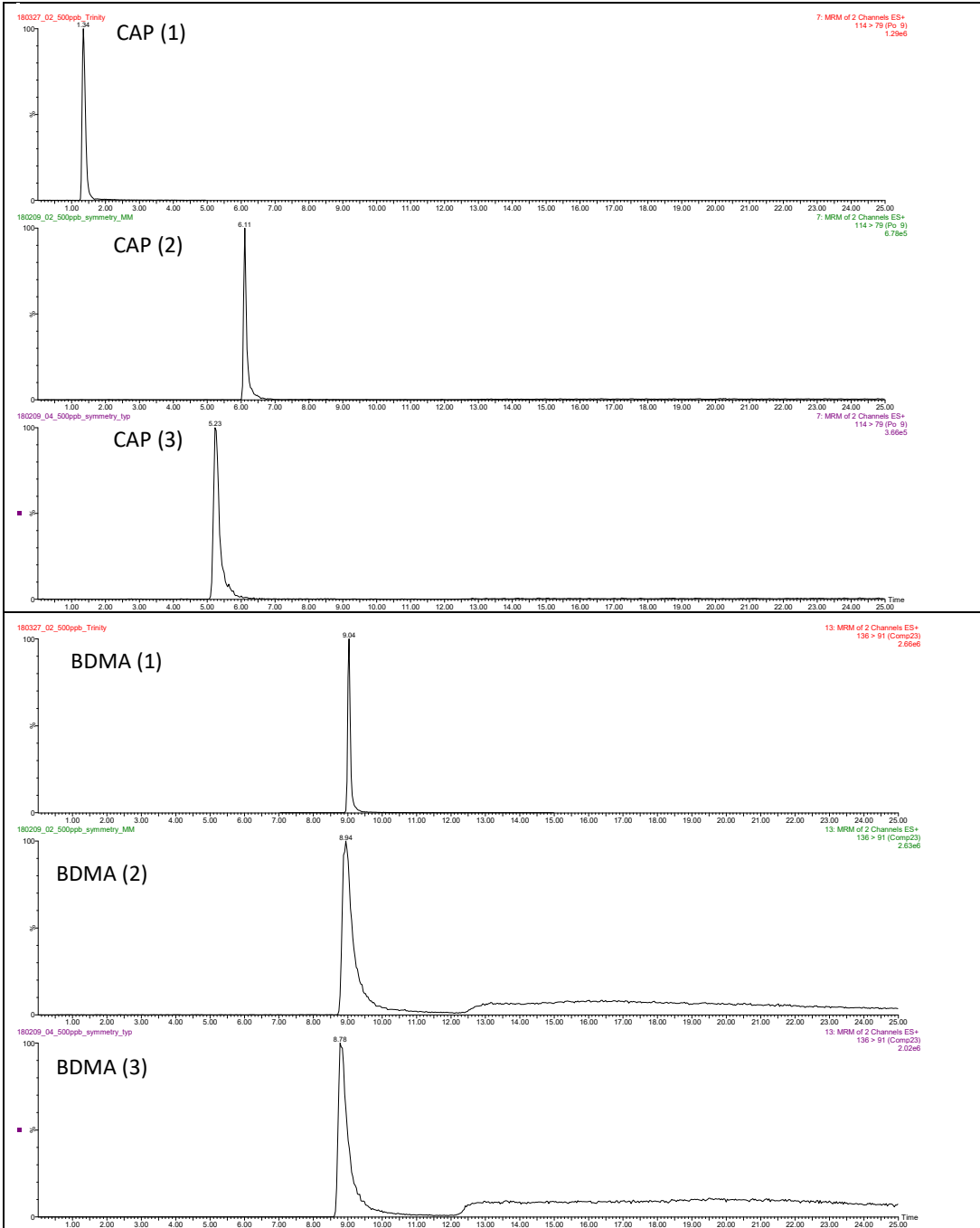
M7 to M9: coastal water.

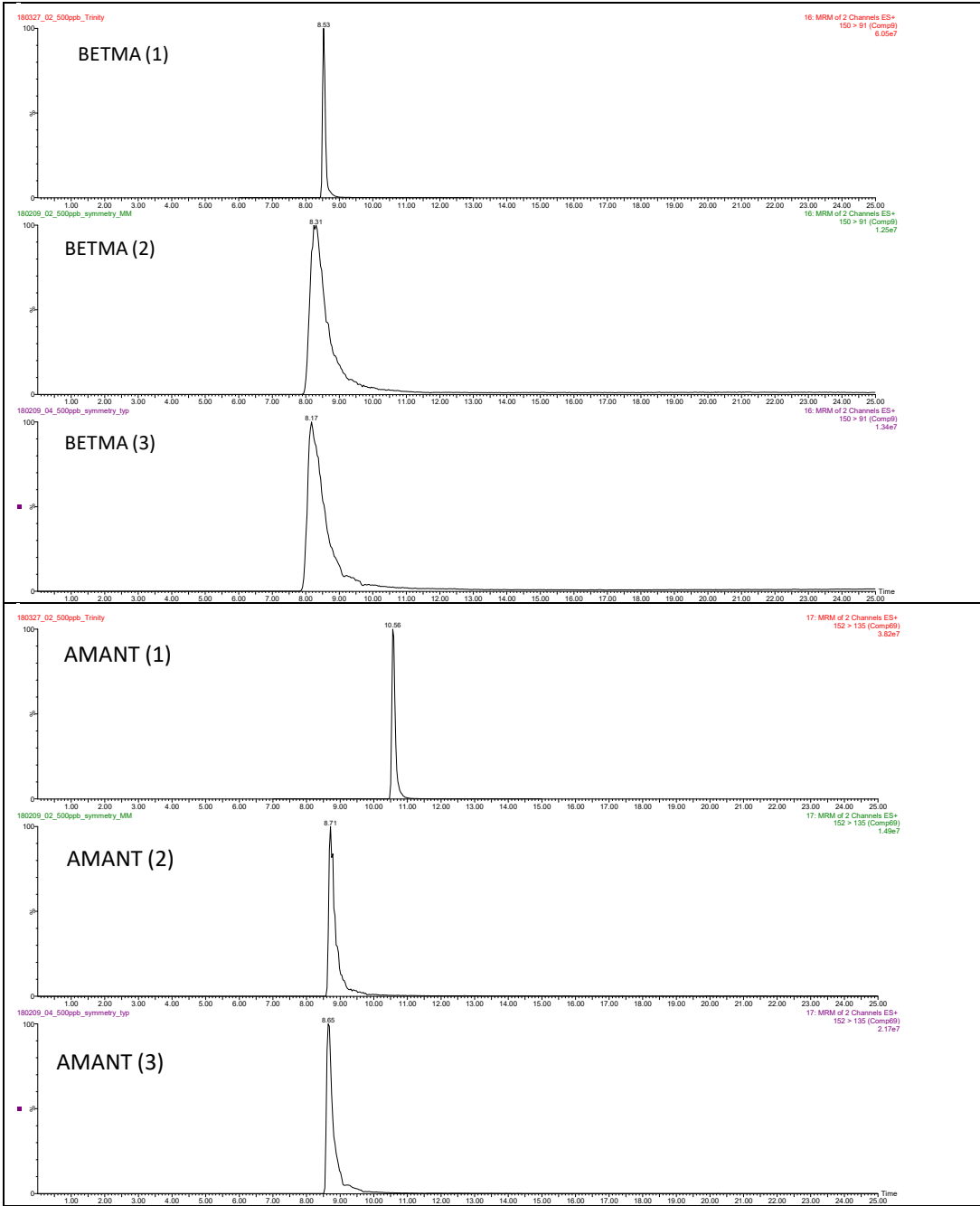
M10 to M12: tap waters collected in the city of Santiago de Compostela (Spain), M10 and M11, and the town of Bertamiráns (Spain), M12.

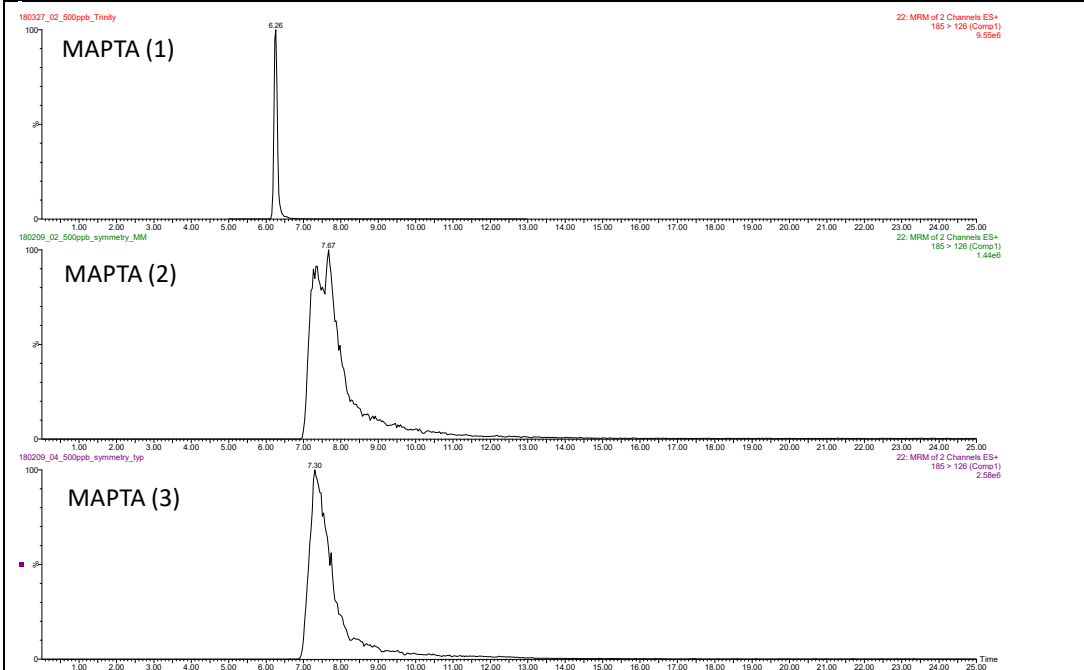
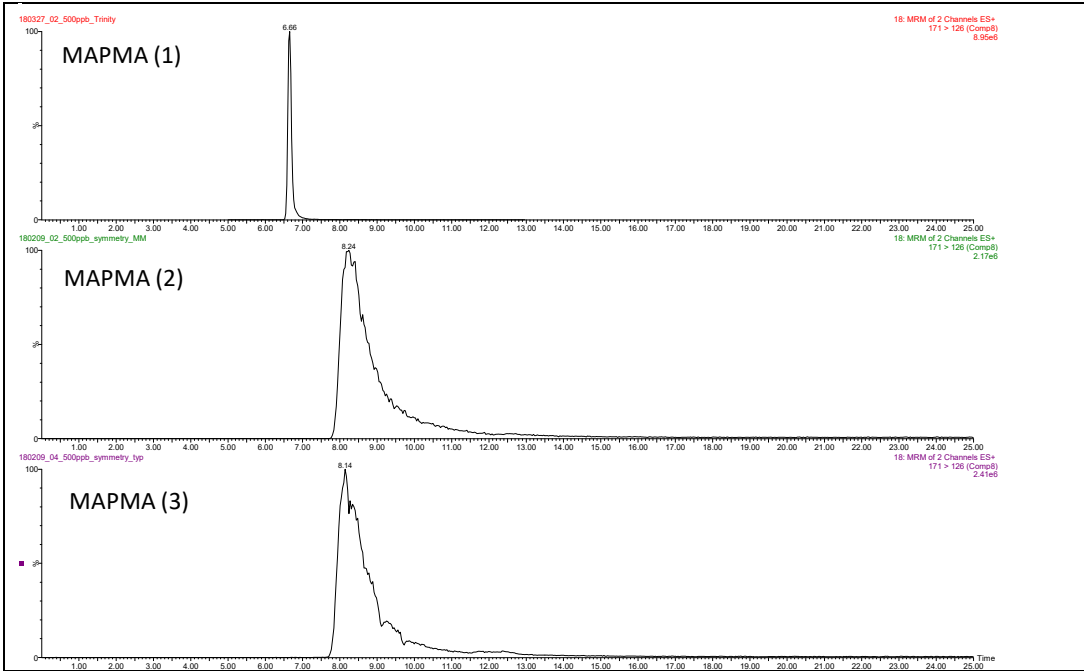
M13 to M15: bottled spring water from three commercial Spanish brands acquired in a local supermarket in Santiago de Compostela (not shown in the map).

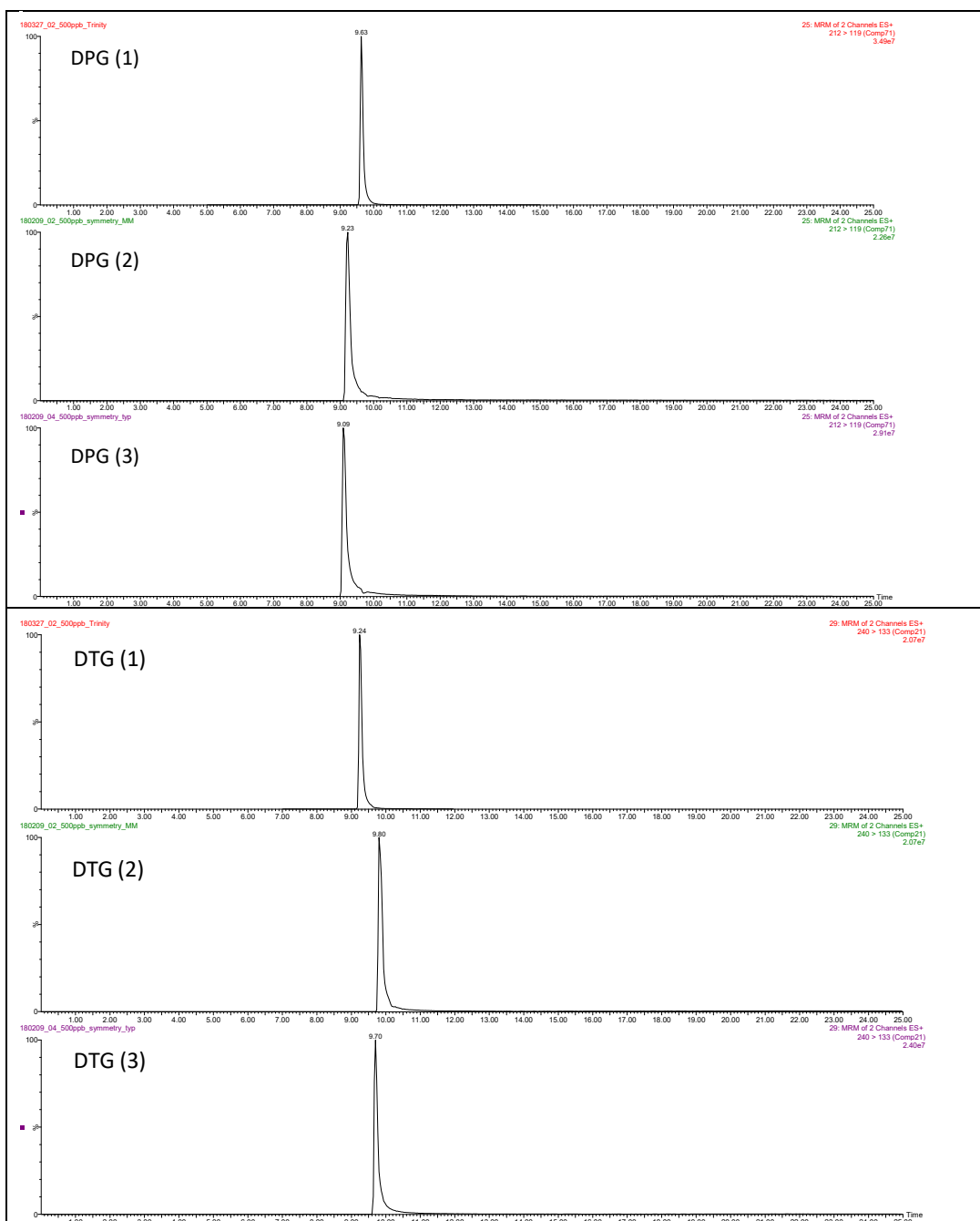
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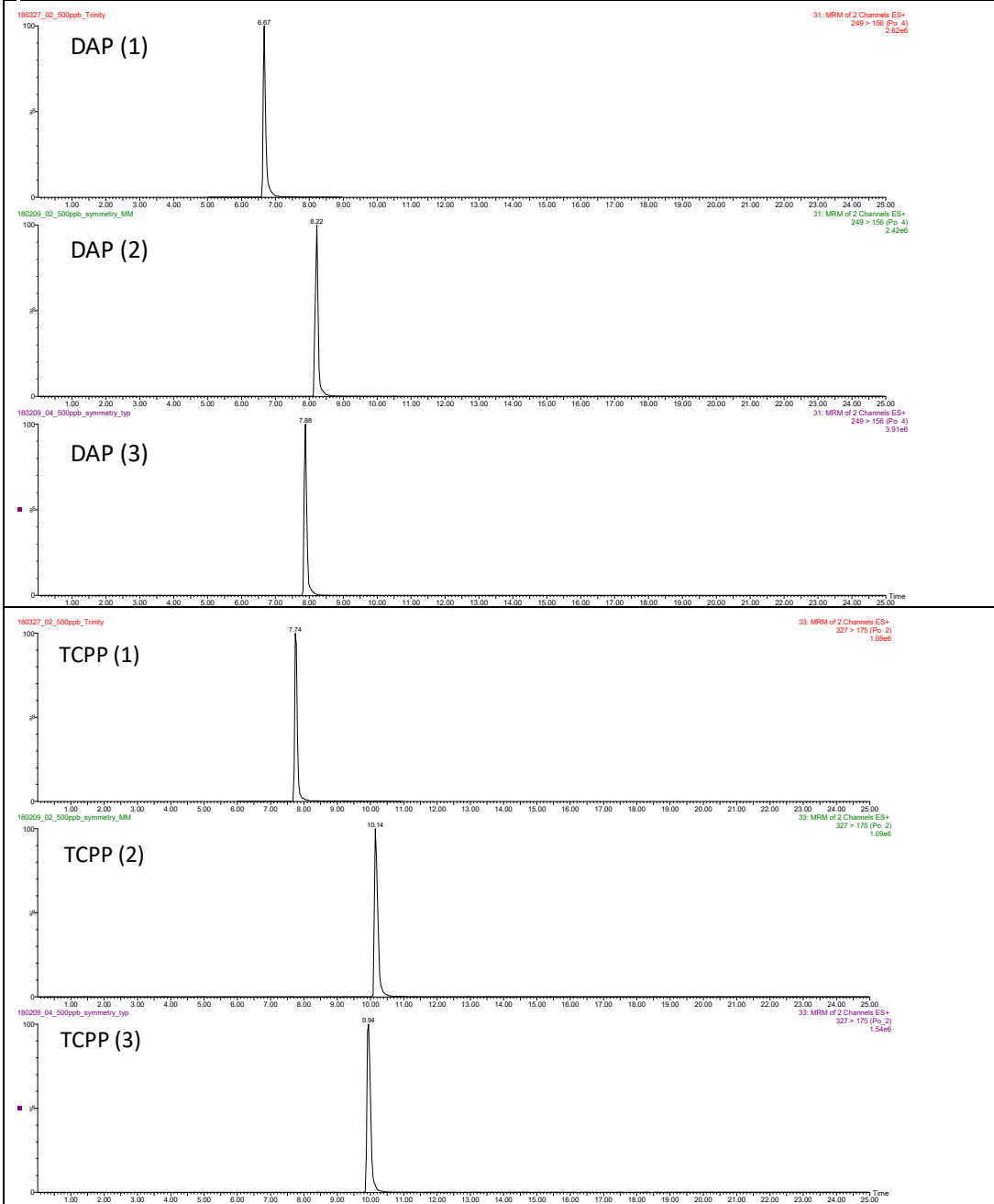


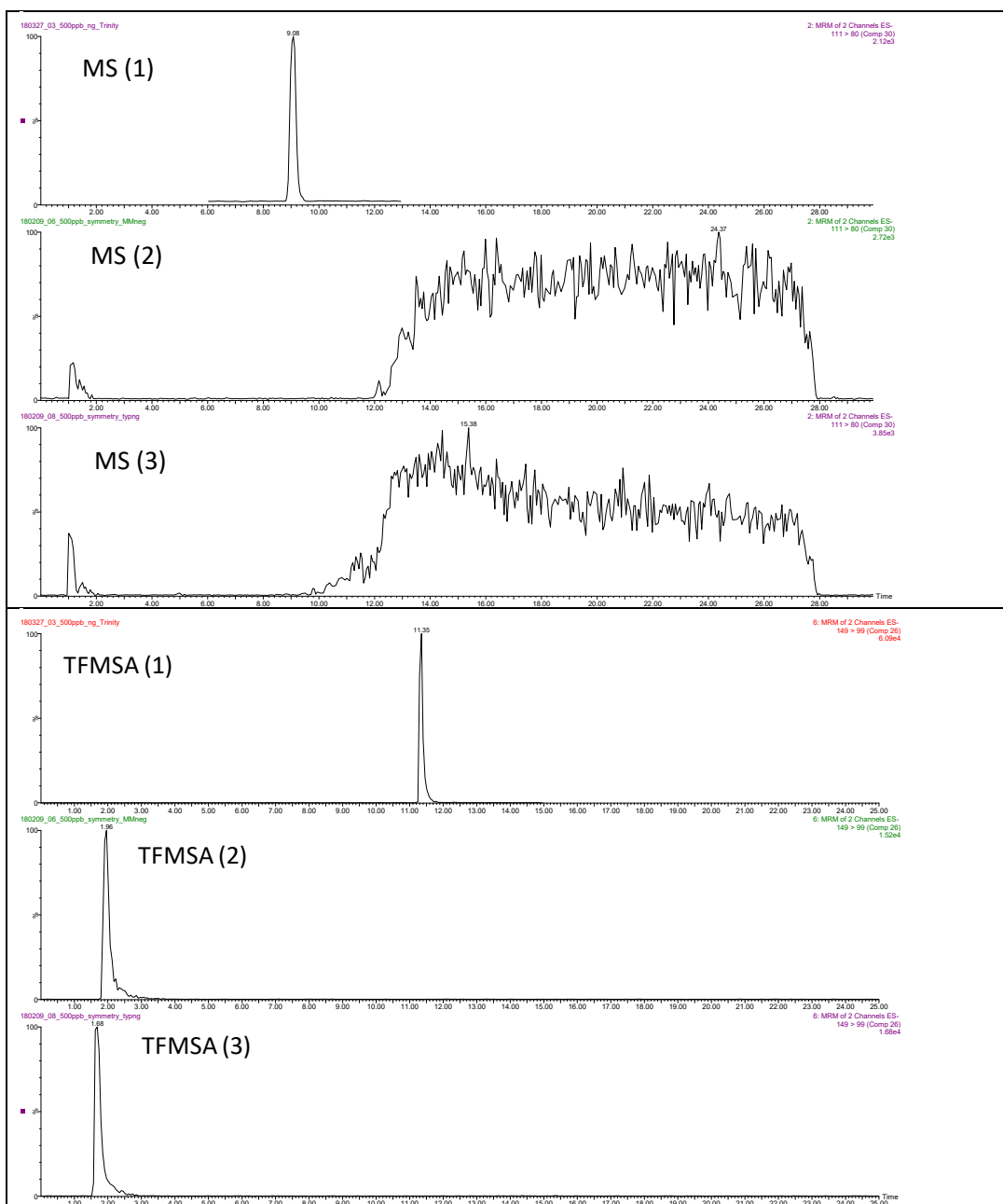


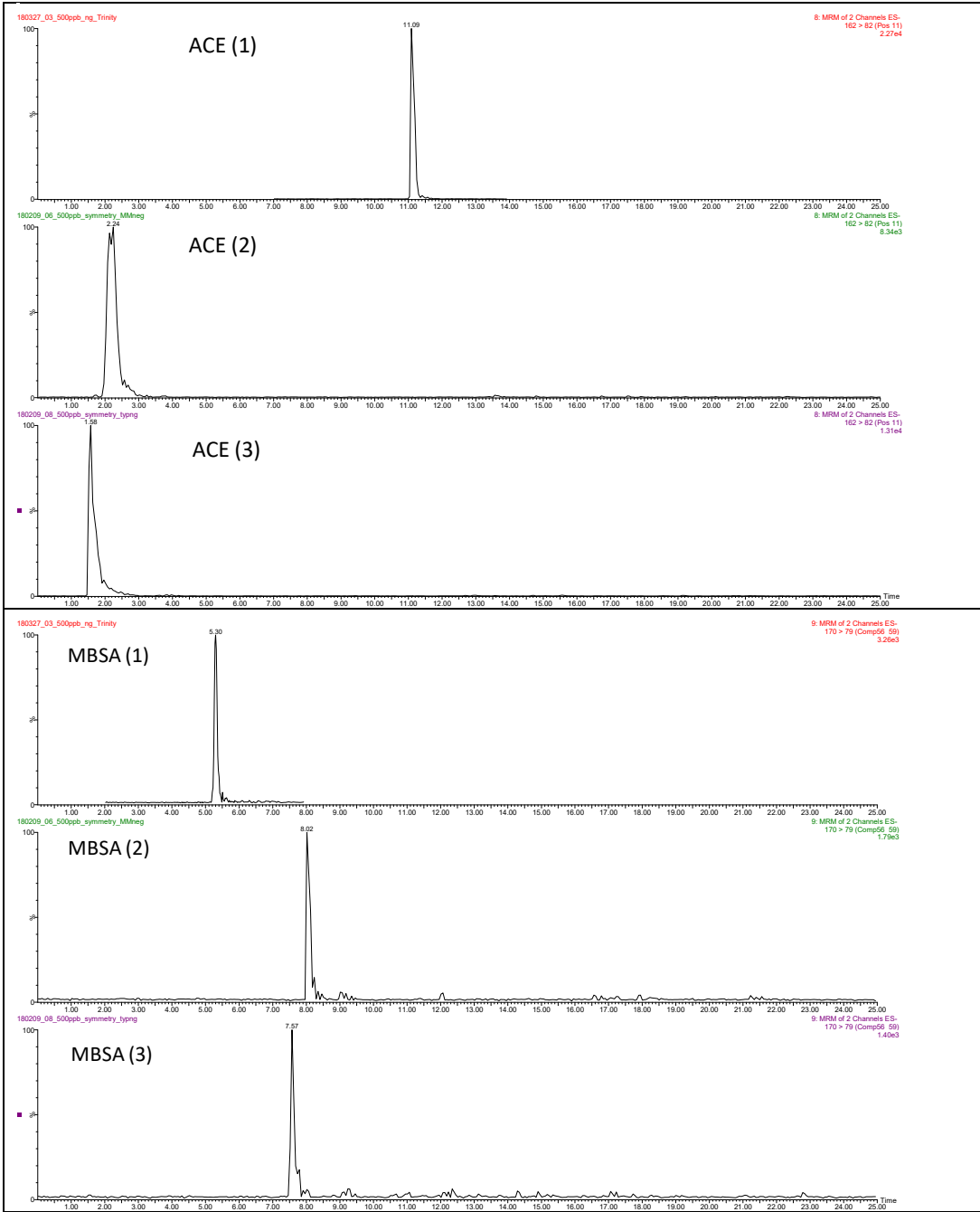


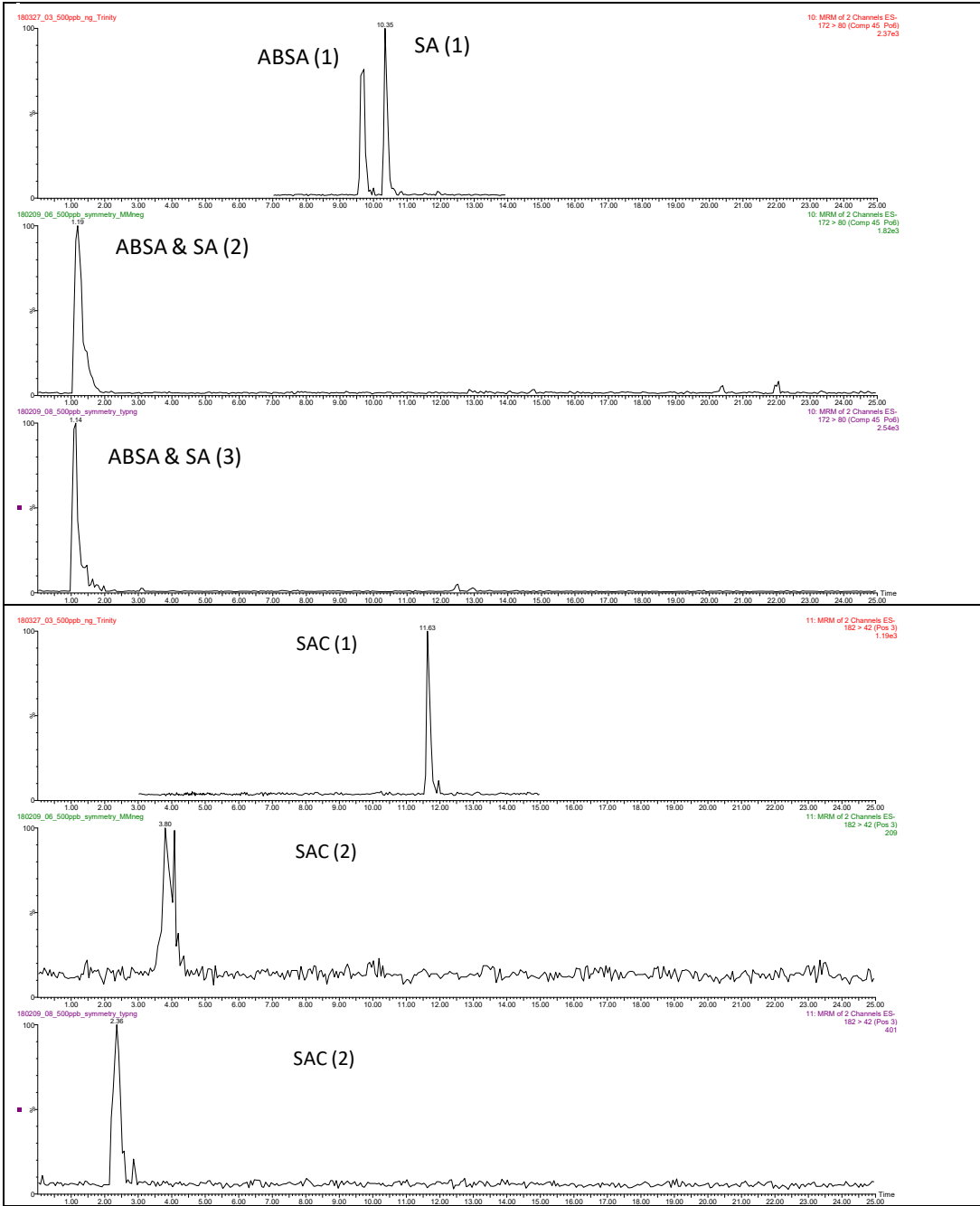


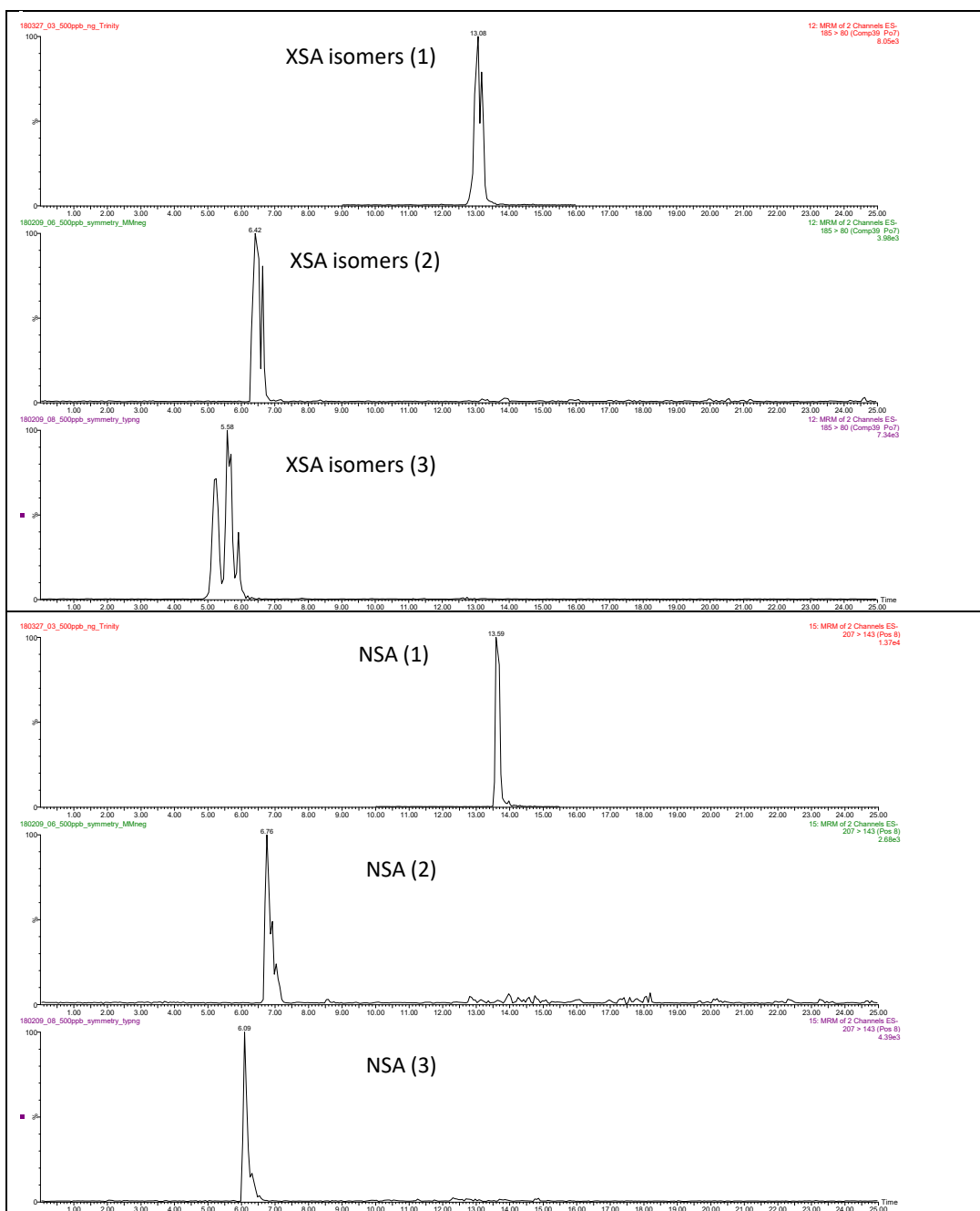












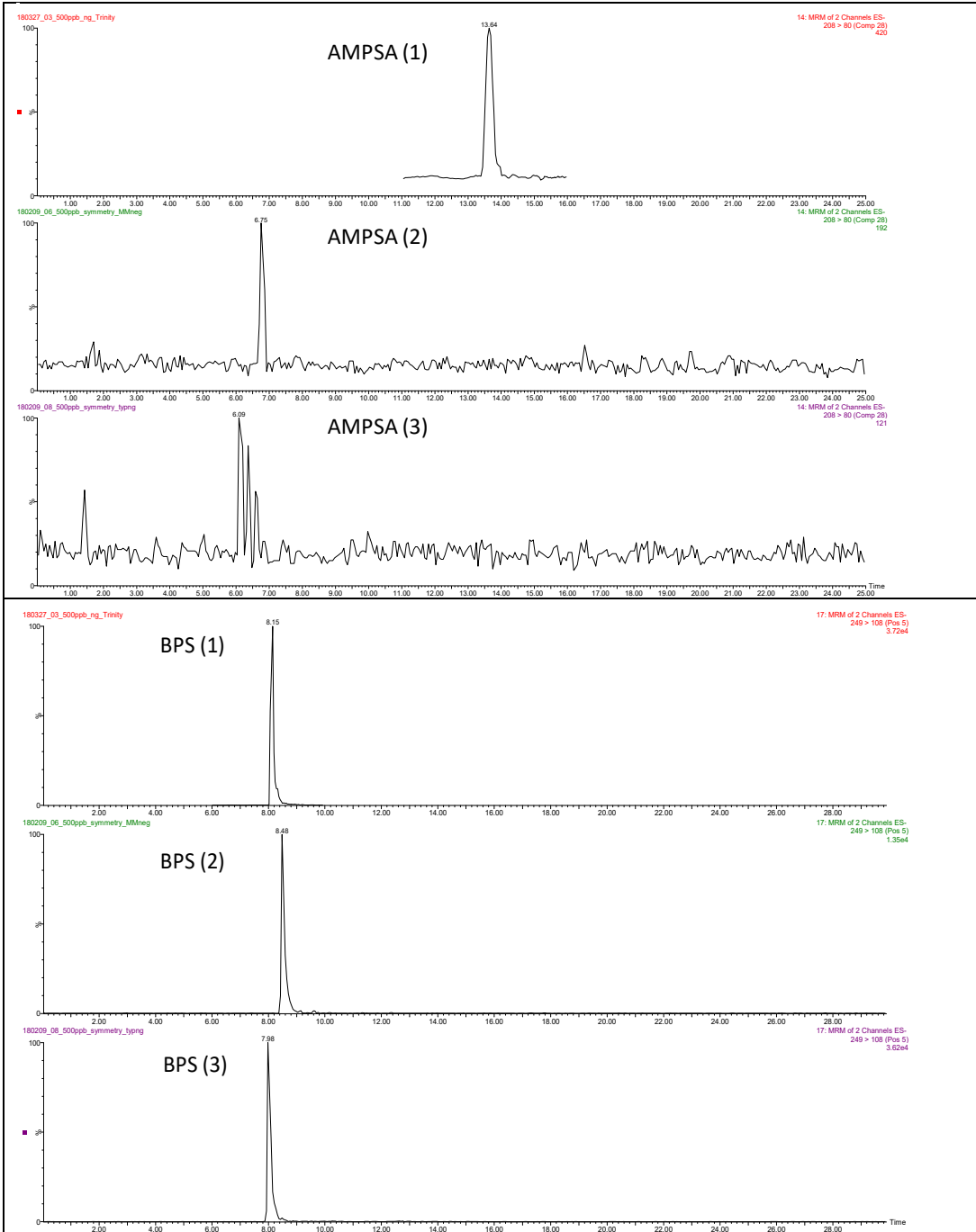


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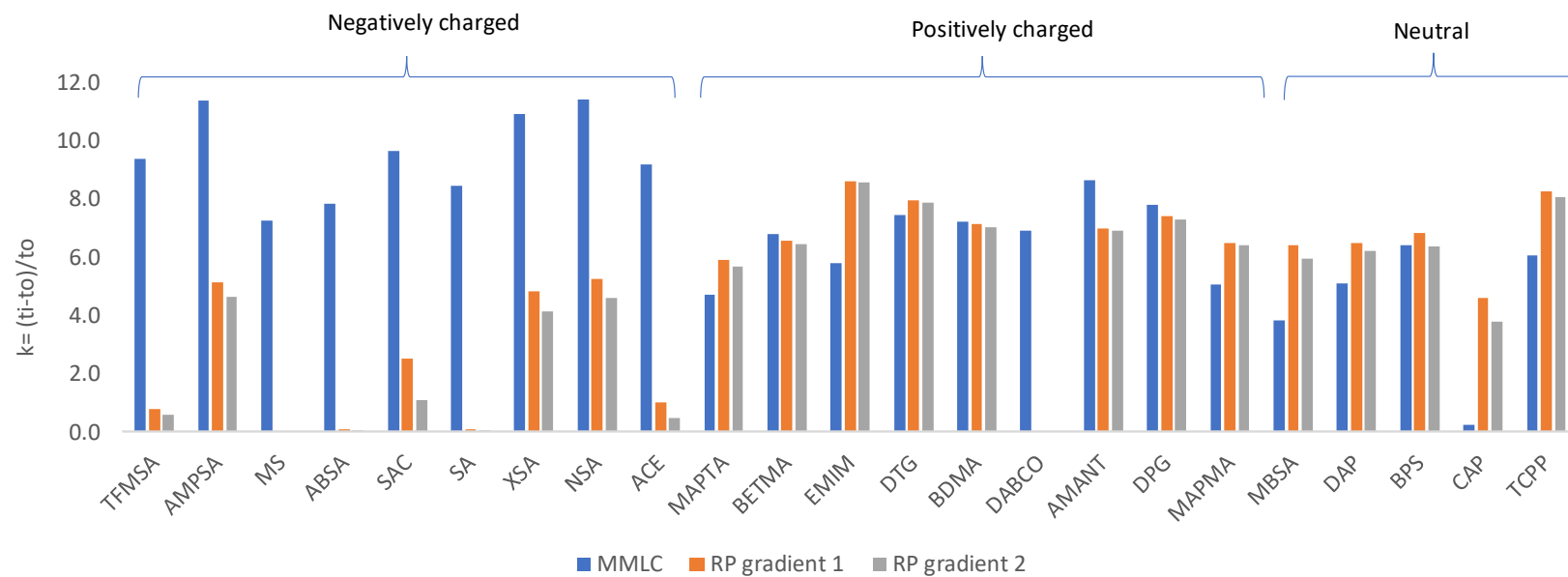


Figure S4: Evaluation of the washing step in SPE protocol. Percentage of analyte found in the washing fraction (6 mL of MeOH) introduced before the final elution (n=3).

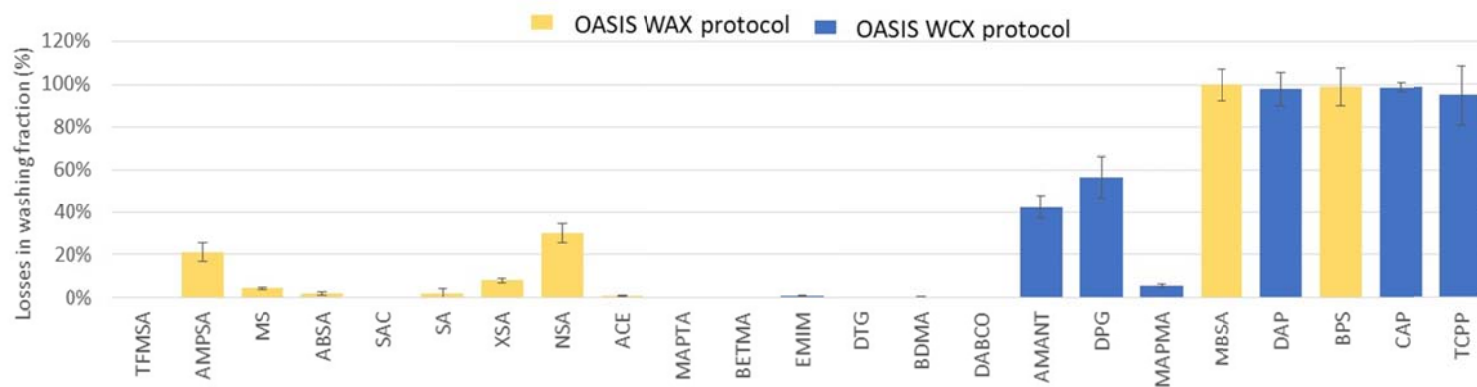


Figure S5: Matrix effects (% ME) of the selected compounds in surface and drinking water (1000 ng L⁻¹ addition level)

