RESEARCH PAPER

Analysis of polar organic contaminants in surface water of the northern Adriatic Sea by solid-phase extraction followed by ultrahigh-pressure liquid chromatography–QTRAP® MS using a hybrid triple-quadrupole linear ion trap instrument

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Abstract Water-soluble polar organic contaminants are discharged by rivers, cities, and ships into the oceans. Little is known on the fate, pollution effects, and thresholds of toxic chemical mixtures in the marine environment. A new trace analytical method was developed for the multi-compound analysis of polar organic chemical contaminants in marine waters. The method is based on automated solid-phase extraction (SPE) of one-liter water samples followed by ultrahigh-pressure liquid chromatography triple-quadrupole linear ion-trap mass spectrometry (UHPLC–QTRAP® MS). Marine water samples from the open Adriatic Sea taken 16 km offshore from Venice (Italy) were analyzed. Method limits of quantification (LOQs) in the low picogram per liter (pg/l) concentration range were achieved. Among the 67 target chemicals analyzed, 45 substances could be detected above the LOQ. The chemicals detected at the highest concentrations were caffeine (up to 367 ng/l), nitrophenol (36 ng/l), 2,4-dinitrophenol (34 ng/l), 5-methyl-1H-benzotriazole (18.5 ng/l), sucralose (11 ng/l), 1H-benzotriazole (9.2 ng/l), terbuthylazine (9 ng/l), alachlor (7.7 ng/l), atrazine-desisopropyl (6.6 ng/l), diethyltoluamide (DEET) (5.0 ng/l), terbuthylazine-desethyl (4.3 ng/l), metolachlor (2.8 ng/l), perfluorooctanoic acid (PFOA) (2.5 ng/l), perfluoropentanoic acid (PFPeA) (2.3 ng/l), linuron (2.3 ng/l), perfluorohexanoic acid (PFHxA) (2.2 ng/l), diuron (2.0 ng/l), perfluorohexane sulfonate (PFHxS) (1.6 ng/l), simazine (1.6 ng/l), atrazine (1.5 ng/l), and perfluorooctane sulfonate (PFOS) (1.3 ng/l). Higher concentrations were detected during summer due to increased levels of tourist activity during this period.

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Introduction

Chemical pollution of surface waters presents a threat to the aquatic environment as it leads to effects such as acute and chronic toxicity to aquatic organisms, accumulation in the ecosystem, and loss of habitat and biodiversity, and it also poses a threat to human health [1]. The Mediterranean Sea is affected by many of the human activities that occur in highly populated areas of this region; such activities can produce chemical contamination and may result in degradation and an increased risk of serious damage to coastal and marine zones. In the future, coastal areas are expected to face even

greater pressures from these sources of activity [2]. Common contributors to the pollution of marine environments are urban or industrial wastewater discharges, agricultural and industrial activities, human settlements, tourism, resource use, and interventions such as infrastructural development and construction [3, 4].

The synthesis and utilization of hundreds of thousands of synthetic chemical compounds in agricultural, industrial, and household applications [5] have perturbed the chemical composition of the biosphere, including ocean waters [6, 7]. Long-range atmospheric and oceanic transport is a significant pathway for the introduction of organic pollutants into remote oceanic regions, leading to their subsequent accumulation in marine organisms [6, 8].

The European Water Framework Directive (WFD) [9] sets environmental quality standards (EQS) for 33 priority substances [10] in inland and coastal surface waters, which are defined as being within a distance of one nautical mile on the seaward side. The EQS are established in the recent Directive 2008/105/EC [1, 11]. According to the WFD, Member States should implement measures with the aim of progressively reducing pollution from priority substances, and ceasing or phasing out emissions, discharges, and losses of priority hazardous substances. Implementation of the WFD involves monitoring and controlling priority pollutant concentrations to ensure the protection of coastal ecosystems [3]. In addition, according to the Marine Strategy Framework Directive (MSFD), concentrations of contaminants in marine waters "should be at levels not giving rise to pollution effects." Member States are required to monitor the concentrations of contaminants in the marine environment, and their effects need to be assessed taking into account their impacts on and threats to the ecosystem [12, 13].

Laboratory and field studies have shown that the combined effects of chemicals upon aquatic life can be additive, resulting in observable detrimental effects of combinations of chemicals, even when each individual chemical is present at a level below which any adverse effects can be detected [14, 15]. Echeveste and coworkers [6] have shown that toxic effects are exerted on phytoplankton abundances by complex mixtures of organic pollutants at concentrations 20–40-fold those found in the open oceans.

However, data and publications on the occurrence of polar organic contaminants in coastal or open marine waters are relatively scarce. Polar chemicals studied in marine waters include antifouling pesticides (e.g., diuron, irgarol) [16, 17], benzotriazoles [18], bisphenol A [19, 20], caffeine [21–24], carbamazepine [21, 22], clofibric acid [23, 25], diclofenac [26], DEET [23, 24], domoic acid [27], estradiol hormones [19], gemfibrozil [26], ibuprofen [24, 26], linear alkylbenzenesulfonates (LAS) [28, 29], naproxen [26], nonylphenols [4, 19, 20, 28–34], nonylphenol ethoxylates [20, 28–30, 32], mecoprop [25], metoprolol [21],

octylphenol [3, 19, 29], organophosphorus flame retardants [35, 36], musk fragrances [35, 37], oxazepam [21], per- and polyfluoroalkyl substances (PFASs) [4, 8, 38–42], sucralose [43], triazine herbicides [22, 44], and triclosan [35, 45].

In addition, hydrophobic pollutants such as toluene, benzene, xylene, ethylbenzene (volatile organic compounds, VOCs), organochlorinated pesticides, phthalates and tributyltin compounds [3], endosulfan [4, 46], polyaromatic hydrocarbons (PAHs) [4], polychlorinated biphenyls (PCBs) [4, 47, 48], hexachlorocyclohexanes (HCHs) [4, 46, 47], hexachlorobenzene (HCB) [47], polychlorinated dioxins [49], brominated flame retardants [50], and dechlorane plus [51] have been analyzed in marine waters.

Liquid chromatography–mass spectrometry (LC-MS) technologies have opened the analytical window to polar chemical compounds within the last 15 years [52]. Quantitative target analysis based on triple-quadrupole tandem LC-MS/MS provides excellent performance because of its inherent selectivity and sensitivity when working in selected reaction monitoring (SRM) mode. Ultrahigh-pressure liquid chromatography (UHPLC)-MS with sub-2-μm particles offers improved resolution, sensitivity, and faster analysis compared to conventional HPLC-MS. The excellent performance of UHPLC-MS/MS has already been shown for multicompound water analysis [53–56]. Some research can be found in the literature on the use of linear ion trap hybrid triple-quadrupole instruments (QTRAP®) coupled to conventional liquid chromatography (LC) [57–59] or UHPLC systems [55] for the trace analysis of pharmaceuticals in environmental waters.

The objective of the study reported in the present paper was to develop a multicompound analytical method for the analysis of polar organic chemical contaminants in marine water samples.

Materials and methods

Chemicals, reagents, and standards

The native chemical standards were purchased from Sigma–Aldrich (St. Louis, MO, USA), Dr. Ehrenstorfer (Augsburg, Germany), Spectra 2000 (Rome, Italy), and Wellington Laboratories (Guelph, Ontario, Canada). In most cases, the standards were supplied in the form of solid crystals, which were weighed and dissolved in methanol (in some cases the addition of water or acidification was required) to prepare a stock standard solution of each target compound at a concentration of approximately 1,000 mg/l which was stored at 4 °C. Working solutions at different concentrations were prepared by appropriate dilution of the stock solutions in methanol.

The following isotopically labeled internal surrogate standards were used for quantification of the water samples: $^{13}C_6$-2,4-D (Cambridge Isotope Laboratories (CIL), Andover, MA, USA), $^{13}C_3$-atrazine (CIL), benzotriazole-d4 (Spectra 2000), bezafibrate-d4 (Spectra 2000), $^{13}C_3$-caffeine (CIL), carbamazepine-d10 (CIL), DEET-d6 (CIL), diclofenac-d4 (Spectra 2000), diuron-d6 (Spectra 2000), gemfibrozil-d6 (CIL), $^{13}C_3$-ibuprofen (CIL), isoproturon-d3 (Spectra 2000), $^{13}C_3$-naproxen (CIL), $^{13}C_4$-PFOA (Wellington Laboratories), $^{13}C_5$-PFNA (Wellington Laboratories), $^{13}C_4$-PFOS (Wellington Laboratories), $^{13}C_3$-simazine (CIL), sucralose-d6 (Spectra 2000), $^{13}C_6$-sulfamethazine (CIL), and $^{13}C_6$-sulfamethoxazole (CIL).

HPLC-grade or analytical grade methanol and acetonitrile were supplied by Merck (LiChrosolv; Darmstadt, Germany) and Sigma–Aldrich (Suprasolv). Water used for the LC-MS/MS analyses was provided by a Milli-Q system (Millipore, Billerica, MA, USA). Acetic acid (TraceSelectUltra for trace analysis, > 99.0 %; Fluka Sigma–Aldrich Group) was also used.

The target analytes included in this study were selected on the basis of previous experience [60–62] and literature studies. They comprise a group of 67 polar organic chemicals belonging to different compound categories: pesticides (and metabolites), pharmaceuticals, benzotriazoles, perfluoroalkyl substances (PFAS), and sucralose (see Table S1 of the "Electronic supplementary material," ESM).

Study area: the northern Adriatic Sea

The Adriatic Sea is a continental basin of the Mediterranean Sea that is located between the Italian Peninsula and the Slovenian–Croatian–Montenegro–Albanian coasts. Its major axis (about 800 km long, versus 200 km for its minor axis) is oriented in the NW–SE direction. The basin has been divided into a northern, a central, and a southern sub-basin. The northern Adriatic Sea is a very shallow basin with an average depth of 35 m. It is characterized by limited circulation of seawater masses which are strongly influenced by atmospheric conditions, mainly winds. The main winds that blow over the Adriatic Sea are the "bora" (a northeasterly wind) and the "scirocco" (a southeasterly wind). In particular, bora events can cause strong heat losses in the northern Adriatic Sea and the formation of the northern Adriatic deep water (NADW) during winter. Another factor influencing the formation of NADW is the water flux that is largely due to runoff from the Po River, which can lower the salinity (and hence the density) of the NADW. The circulation in the northwestern Adriatic is mainly influenced by the coastal southward flow of the North Adriatic current and a North Adriatic (cyclonic) gyre in autumn. The northern Adriatic Sea is strongly influenced by the discharge from the Po River, which markedly increases its biological productivity compared to the oligo-trophic characteristics of the Mediterranean Sea and most of the Adriatic Sea [63].

Large amounts of fresh water from the Po River and other northern Italian rivers enter the marine system in the Adriatic Sea along the northern and northwestern coasts. The Po River represents the major source of fresh water, providing about 50 % of the total water mass load at an annual mean discharge rate of 1,500–1,700 m^3/s, which accounts for about one-third of the total riverine freshwater input into the Adriatic Sea [64]. Other important rivers are the Adige (median discharge flow: 235 m^3/s), Brenta (93 m^3/s), Piave (125 m^3/s), Tagliamento (70 m^3/s), Isonzo (Soĉa in Slovenian) (170 m^3/s), and Reno (95 m^3/s), south of the Po River (Fig. 1). The salinity distribution can show strong seasonal fluctuations during the year, characterized by freshwater inputs from the Po River during strong rainfall events, which usually occur in spring and autumn and can cause large decreases in salinity from the usual level of 35 g/l down to 15 g/l. The water column is generally well mixed from November through February [64–66].

The middle Adriatic Sea (average depth: about 140 m) is characterized by two depressions at the bottom (the so-called Pomo or Jabuka pits) that can be about 250 m deep. The southern Adriatic is characterized by a strong topographic gradient that leads to a wide depression with a maximum depth of about 1,200 m [63].

Water sampling from the Acqua Alta Oceanographic Tower (Venice, Italy)

The volume of each (grab) water sample taken was one liter. One-liter Schott Duran glass bottles were used for sample storage and transport. These bottles were cleaned beforehand in the laboratory in a dishwasher and cooked in an oven overnight at 450 °C. Aluminum foil was inserted into the taps to avoid any contamination. Duplicate 1-l samples were taken in all cases. The samples were shipped in thermostatic boxes cooled with freezing elements, and stored in the laboratory in a fridge at 4 °C until further processing (extraction). The water samples were taken 16 km offshore from Venice (Italy) at the Acqua Alta Oceanographic Tower (http://www.ismar.cnr.it/infra-structure/piattaforma-acqua-alta; 45°18′51″N; 12°30′30″E) in the northern Adriatic Sea, where the water has a depth of 16 m. The samples were taken on 10 February 2011 (16:15 h; water temperature: 7.2 °C; salinity: 33.8 g/l), 6 May 2011 (9:15 h; water temperature: 16.2 °C; salinity: 33.9 g/l), 15 September 2011 (8:45 h; water temperature: 26 °C; salinity: 34.8 g/l), and 13 March 2012 (11:10 h; water temperature: 8.4 °C; salinity: 38.2 g/l), using a Niskin bottle sampler, which was immersed approximately 50 cm below the water surface.

Fig. 1 Map of the northern Adriatic Sea showing the position (and an image) of the Acqua Alta Oceanographic Tower (sampling point)
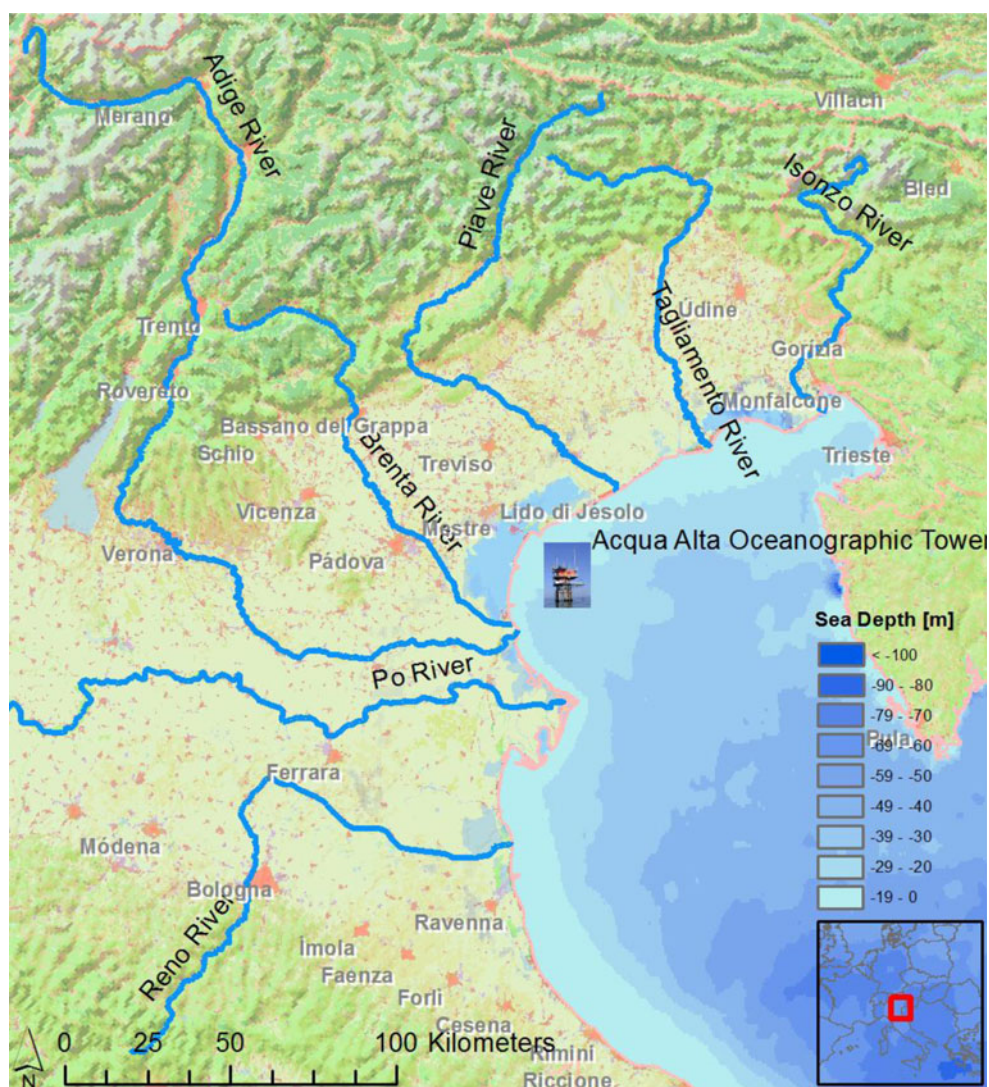
Solid-phase extraction (SPE)

The water samples were extracted by automated solid-phase extraction (SPE) with Oasis HLB (200 mg; 6 ml) hydrophilic–lipophilic balance cartridges using a Gilson (Middleton, WI, USA) ASPEC® 274 robotic system; the extraction volume was 1 l and the water was not filtered. Before extraction, the samples were spiked with the internal (surrogate) standard, which contained the isotopically labeled standards mentioned above. The spiking levels in the water samples were 1 ng/l for $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, and $^{13}C_4$-PFOS and 10 ng/l for the other labeled compounds. The cartridges were activated and conditioned with 5 ml methanol and 5 ml Milli-Q water at a flow rate of 3 ml/min. The water samples (975 ml; 25 ml were pumped through the system after conditioning the cartridges to fill the void volume) were passed through the wet cartridges at a flow rate of 5 ml/min. After extraction, the columns were rinsed with 20 ml Milli-Q water (flow rate 3 ml/min), and the cartridges dried for 12 min using nitrogen at approximately 0.6 bars. Elution was performed with 6 ml methanol (flow rate 1.5 ml/min). Evaporation of the extracts with nitrogen to 200 µl was performed at a temperature of 40 °C in a water bath using a TurboVap® II Concentration Workstation (Caliper Life Sciences, Hopkinton, MA, USA). The overall enrichment factor was 4,875 (extraction of 975 ml, and concentration to 200 µl).

Liquid chromatography QTRAP® mass spectrometry

In this study a new Acquity® ultra performance™ liquid chromatography (UPLC) system (Waters Corporation, Milford, MA, USA) coupled to a hybrid triple-quadrupole linear ion trap mass spectrometer (5500 QTRAP®) with a turbo ion spray source from AB SCIEX (Foster City, CA, USA) were used.

The QTRAP® system was operated for quantification of the target analytes in selected reaction monitoring (SRM)

Table 1 Results from an analysis of polar organic contaminants in open-water samples from the Adriatic Sea (offshore from Venice)

Compounds	10 February 2011	6 May 2011	15 September 2011	13 March 2012	LOQ (average)
Water temperature (°C)	7.2	16.2	26.0	8.4	
Salinity (g/l)	33.8	33.9	34.8	38.2	
1*H*-Benzotriazole	2.927	9.203	8.345	3.750	0.171
5-Methyl-1*H*-benzotriazole	6.425	18.50	17.94	3.112	0.113
Alachlor	< LOQ	< LOQ	7.701	0.437	0.029
Atenolol	0.752	0.174	< LOQ	0.081	0.075
Atrazine	0.589	1.522	1.493	1.031	0.049
Atrazine-desethyl	< LOQ	0.058	0.086	0.047	0.036
Atrazine-desisopropyl	0.543	6.573	3.820	0.761	0.026
Caffeine	132.1	366.9	336.1	82.00	9.104
Carbamazepine	0.188	0.286	0.355	0.106	0.007
Carbendazim	0.187	0.962	1.130	0.165	0.007
Chloridazon	0.160	0.750	1.253	0.662	0.090
DEET	0.349	1.255	4.995	0.460	0.213
Diuron	0.485	1.957	1.948	0.401	0.012
Irgarol (cybutryne)	n.a.	< LOQ	0.131	0.066	0.007
Isoproturon	0.015	0.035	< LOQ	< LOQ	0.010
Linuron	< LOQ	< LOQ	2.301	0.807	0.565
Metolachlor	0.326	2.234	2.799	1.740	0.239
Metoprolol	n.a.	n.a.	n.a.	0.685	0.234
Simazine	0.438	1.566	1.046	0.737	0.203
Sulfadimethoxine	< LOQ	< LOQ	< LOQ	0.087	0.087
Sulfamethoxazole	0.405	1.017	0.349	0.265	0.015
Terbutryn	0.115	0.452	0.209	0.548	0.129
Terbuthylazine	0.774	8.895	5.502	1.555	0.104
Terbuthylazine-desethyl	1.300	4.279	3.734	0.945	0.318
2,4-D	0.244	0.162	0.148	0.090	0.187
2,4-Dinitrophenol	12.94	34.11	7.855	27.10	2.813
2,4,5-T	0.046	0.191	0.219	0.267	0.032
Bentazone	0.068	0.150	0.071	0.075	0.022
Bezafibrate	0.109	0.138	0.140	0.021	0.024
Clofibric acid	< LOQ	0.186	0.182	< LOQ	0.025
Dichlorprop	< LOQ	< LOQ	0.200	< LOQ	0.054
Gemfibrozil	0.110	0.180	0.075	0.024	0.083
Ibuprofen	0.769	1.146	0.113	< LOQ	0.049
MCPA	0.030	0.256	0.427	0.027	0.013
Mecoprop	0.029	0.240	0.125	0.018	0.032
Nitrophenol	5.432	35.69	6.074	28.71	6.732
Sucralose	4.444	9.800	11.08	3.038	0.221
PFPeA	n.a.	n.a.	2.349	n.a.	0.266
PFHxA	n.a.	n.a.	2.202	0.368	0.490
PFHpA	n.a.	0.328	0.298	< LOQ	0.098
PFOA	n.a.	1.323	2.505	0.793	0.351
PFNA	n.a.	n.a.	0.152	0.067	0.209
PFDA	n.a.	n.a.	0.019	< LOQ	0.019
PFHxS	n.a.	n.a.	1.649	n.a.	0.083
PFOS	n.a.	1.343	1.338	n.a.	0.086

Concentrations in ng/l, *n.a.* not analyzed, *LOQ* limit of quantification

acquisition mode (MS/MS) with both positive and negative electrospray ionization. Unequivocal identification was provided by the acquisition of two SRM transitions per compound in most cases (Table S1 of the ESM). The protonated or deprotonated molecular ion of each compound was chosen as the precursor ion.

Separations of the analytes were performed with an UPLC™ system (Acquity® series, Waters) equipped with a quaternary pump, using a reversed-phase BEH C18 analytical column (Waters; particle size 1.7 μm, 50 mm in length, and 2.1 mm i.d.). For the analyses performed in both positive and negative modes, the compounds were separated using Milli-Q water/methanol 95:5 % (v/v), with 0.1 % acetic acid employed as mobile phase A and methanol/acetonitrile 50:50 % (v/v) with 0.1 % acetic acid as mobile phase B at a flow rate of 0.6 ml/min. The gradient elution started with 90 % mobile phase A, which was ramped within 5 min to 90 % mobile phase B, held for 0.5 min, and then reverted to initial conditions via a 0.5 min ramp, allowing 3 min of stabilization time. The total analysis time was 10 min.

To eliminate instrumental blank contamination, the Acquity® UPLC™ system was modified with an Acquity® PFC analysis kit, containing PEEK solvent tube lines, stainless steel tubing, filters, and a PFC isolator column. The PFC isolator column was placed in-line between the solvent mixer and the injector. It eliminated background PFAS (perfluoroalkyl substances) interference from the UPLC™ system (PTFE tubes, fittings, and connections, etc.).

The operating conditions for the analyses performed in both positive and negative ionization modes were as follows: ion spray voltage 4,500 V; curtain gas 25 (arbitrary units); ion source gases GS1 and GS2 were 55 and 45 psi, respectively; probe temperature 550 °C. Nitrogen served as nebulizer and collision gas.

Careful optimization of the compound-dependent MS parameters was performed for each chemical substance. Sensitivity and precision were the main objectives of the optimizations. SRM experiments were carried out to determine the maximum sensitivity for the detection of the target molecules. Optimization of MS parameters [declustering potential (DP) and collision energy (CE)] was performed by flow injection analysis for each compound. The entrance potential (EP) for precursor ions and the collision cell exit potential (CXP) for product ions were not changed for any of the compounds because they had very little influence on the optimization process. They were set to default values of 10 (EP) and 11 (CXP). The declustering potential was optimized for each compound in order to obtain the maximum response for the protonated $[M+H]^+$ or deprotonated $[M-H]^-$ molecular ion and to prevent in-source fragmentation or adducts. Collision energies and collision cell

entrance and exit potentials were optimized in order to obtain the two most sensitive transitions. The most intense one was used for quantification (quantifier), whilst the other was used for confirmation purposes (qualifier). Table S1 of the ESM summarizes the precursor ions and suitable MS/MS transitions selected by the optimization procedure. All data were acquired and processed using the Analyst® 1.5.1 software package.

Identification, quantification, and QA/QC

Quality assurance and quality control (QA/QC) included the use of isotopically labeled internal standards, the determination of recovery rates and of method, instrumental, and field blanks, as well as reproducibility experiments (analysis of duplicate samples). The compounds were identified by matching retention times and their specific MS/MS SRM transitions and quantifier to qualifier ion ratios (see the tables in the ESM). Quantification of the individual chemicals was performed with the corresponding or similar isotopically labeled surrogate standards (IS); identical IS were not available for all individual chemicals (see Table S1 of the ESM). The relative response factors of the compounds in relation to the IS were calculated in all cases. Thus, the reported concentrations are corrected for the exact recoveries only for the compounds for which identical IS were available. The uncertainties in the concentrations of the other target compounds are a bit higher due to different recoveries.

Linear response ranges covering three orders of magnitude (10–1,000 μg/l) were demonstrated for all compounds ($R^2 > 0.99$). The method detection limits or limits of detection (LODs) for the SPE-LC-MS^2 procedure were calculated according to ISO/TS 13530 [67] as three times the standard deviation of the blank samples. The limits of quantification (LOQs) are usually 3 times the LOD (LOQ = 3 × LOD). However, it should be noted that the LOD (or LOQ) is not a constant value, because it depends on blank contamination and the (sensitivity) performance of the instrument. Moreover, in some cases it was not possible to "quantify" blanks from the baseline noise of the detector (Table S3 of the ESM). Measurement uncertainty is estimated to be around 25–50 %. The highest uncertainty contribution stems from recovery variations for analytes and surrogate standards (no recovery correction was performed) and the standard preparation process. The recovery studies ($n = 2–4$) were carried out by spiking Milli-Q water at realistic concentration levels of 1–100 ng/l with a mixture of the native and labeled target compounds. External quantification was performed in this case. The relative standard deviations (RSDs) were generally below 20 %. Moreover, for additional QA/QC, our

laboratory regularly participates in interlaboratory studies of water analysis. More information on analytical precision concerning the quantification of both MS/MS transitions and the standard deviations of the duplicate samples analyzed is given in the ESM.

Results and discussion

UHPLC-QTRAP® MS

In order to optimize the chromatographic separation, different mobile phases were tested. Acetic acid was used as an additive because it has already shown good performance [60–62]. Initially, a mobile phase consisting of water (A) and acetonitrile (B) was selected (both containing 0.1 % acetic acid), but better chromatographic peaks were achieved with mixtures of water and methanol (95:5 %, v/v) with 0.1 % acetic acid (mobile phase A) as well as methanol and acetonitrile (50:50 %, v/v) with 0.1 % acetic acid (mobile phase B). A linear gradient from 90 % to 10 % of A in 5 min and a flow rate of 0.6 ml/min was applied.

Ultrahigh-pressure LC (UHPLC) systems with sub-2-μm particles allow chromatographers to work at higher efficiencies with much wider ranges of linear velocities, flow rates, and backpressures than are possible with HPLC. A UHPLC multi-compound separation of the 67 target analytes chosen (Table S1 of the ESM) was achieved in just 10 min of analysis time. Target analysis of contaminants by UHPLC-QTRAP®-MS/MS provided accurate quantitative results for the target compounds. Very low method limits of quantification (LOQs) in the picogram per liter (pg/l) concentration range were achieved for many chemicals with 1-l water samples (Tables S1 and S4 of the ESM). However, some very polar substances, such as acesulfame, saccharin, perfluorobutanoic acid (PFBA), and perfluorobutane sulfonate (PFBS), could not be included in the final multi-compound analytical method due to low recoveries and/or elution that was too fast on the chromatographic column. Also, the detection of atenolol was difficult due to a low retention time (0.50 min; Fig S5 of the ESM).

Figure 2 shows exemplary SRM chromatograms for two chemicals, 1H-benzotriazole (8.35 ng/l) and carbamazepine (0.286 ng/l), that were detected in the Adriatic Sea, and corresponding blank samples (below). Very sharp peaks were obtained for most compounds with the UHPLC-MS/MS method developed here. The LOQs calculated from the blank levels of 1H-benzotriazole (0.010 ng/l) and carbamazepine (0.004 ng/l) were 0.051 and 0.010 ng/l, respectively. The SRM chromatograms for the other compounds detected are given in the supporting information (Figs. S1–S46 of the ESM).

Solid-phase extraction

Solid-phase extraction (SPE) was performed with hydrophilic–lipophilic balance (HLB) cartridges, which are well suited to the extraction of polar water-soluble compounds from water. The objective of this study was to develop an easy-to-use SPE method for marine water samples. The pH of the water samples was not changed. After extracting marine water samples, it is important to wash the cartridges with water (Milli-Q) to remove the salt from the extracts; this reduces LC-MS matrix effects (ion suppression) [26]. The recoveries, which are given in Table S1 of the ESM, were >50 % in most cases. Moreover, it is very important to dry the cartridges well with nitrogen gas before the elution. Incomplete drying could cause lower recoveries for some compounds. Apparently, recovery problems were encountered for PFHxS, PFOS, and $^{13}C_4$-PFOS in the samples from 13 March 2012 due to incomplete drying of the cartridges. Therefore, PFHxS and PFOS could not be analyzed in these samples.

Chemicals detected in the northern Adriatic Sea

We analyzed four open-water samples taken 16 km offshore from Venice in the Adriatic Sea (in duplicate) at the Acqua Alta Oceanographic Tower for polar organic chemicals. The analytical results are presented in Table 1 and Fig. S1 of the ESM, and more details are provided in Table S2 of the ESM. In general, lower concentrations were found in the samples taken in February 2011 and March 2012 compared to those in May and September 2011. This difference can be explained by general variations in currents or mixing of water masses, but also it appears that higher contamination levels were detected during summer. This can be explained by the much higher levels of tourist activity that occur in summer (from May to September) in many places in the Adriatic. Salinity is not a good indicator of the concentration levels of the organic contaminants investigated. Higher salinity means less of an influence of fresh water from rivers, which would lead us to expect lower levels of contaminants. The lowest salinity, however, was not observed in the samples from May and September, in which the highest contaminant levels were detected (Table 1).

The chemical compounds detected at the highest concentration levels in the Adriatic Sea were caffeine (82–367 ng/l), followed by 2,4-dinitrophenol (7.9–34 ng/l), nitrophenol (5.4–35.7 ng/l), and the benzotriazoles, a class of high-production-volume chemicals with a broad range of applications in various industrial processes as well as in households, mainly as corrosion inhibitors
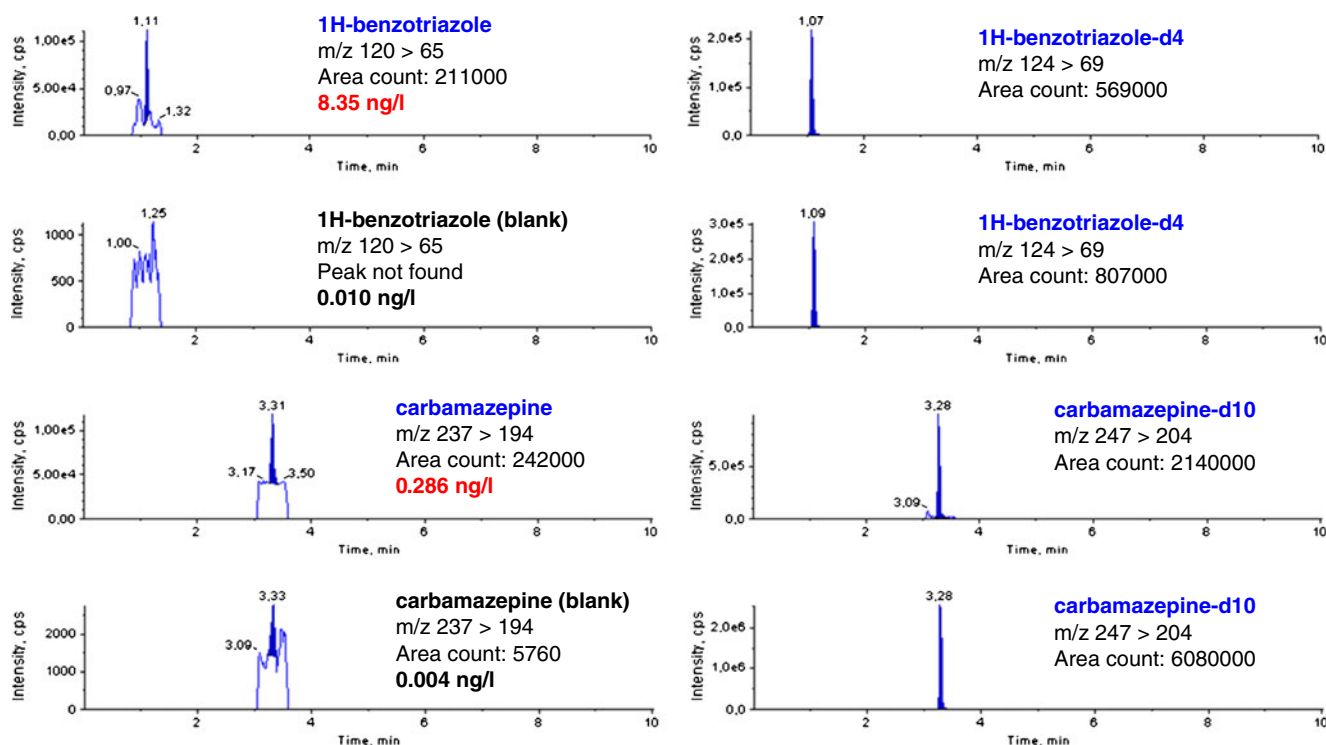
Fig. 2 SRM chromatograms of 1*H*-benzotriazole and carbamazepine in the Adriatic Sea and in blank samples. Sample dates: 15 September 2011 (1*H*-benzotriazole) and 6 May 2011 (carbamazepine). LOQs: 0.051 ng/l for 1*H*-benzotriazole and 0.010 ng/l for carbamazepine

([18], and references therein). In the northern Adriatic Sea, 3.1–18.5 ng/l 5-methylbenzotriazole (or tolyltriazole) and 2.9–9.2 ng/l 1*H*-benzotriazole were detected. Unfortunately, we only had a chemical standard for 5-methyl-benzotriazole, not for 4-methylbenzotriazole.

Other chemicals detected in the marine waters of the Adriatic Sea at relatively high levels (above 1 ng/l) were sucralose (3.0–11.1 ng/l), terbuthylazine (0.77–8.90 ng/l), atrazine-desisopropyl (0.54–6.57 ng/l), DEET (0.35–5.00 ng/l), terbuthylazine-desethyl (1.30–4.28 ng/l), alachlor (<0.029–7.70 ng/l), metolachlor (0.33–2.80 ng/l), perfluorooctanoic acid (PFOA) (0.79–2.51 ng/l), perfluoropentanoic acid (PFPeA) (2.35 ng/l), perfluorohexanoic acid (PFHxA) (0.37–2.20 ng/l), linuron (<0.57–2.30 ng/l), perfluorohexane sulfonate (PFHxS) (1.65 ng/l), diuron (0.40–1.96 ng/l), perfluorooctane sulfonate (PFOS) (1.34 ng/l), atrazine (0.59–1.52 ng/l), simazine (0.44–1.57 ng/l), chloridazon (0.16–1.25 ng/l), carbendazim (0.17–1.13 ng/l), ibuprofen (0.11–1.15 ng/l), and sulfamethoxazole (0.27–1.02 ng/l) (see Table 1).

Chemicals detected at lower levels were atenolol (<0.075–0.75 ng/l), perfluoroheptanoic acid (PFHpA) (0.30–0.33 ng/l), carbamazepine (0.11–0.36 ng/l), terbutryn (0.12–0.55 ng/l), MCPA (0.027–0.43 ng/l), 2,4-D (0.090–0.24 ng/l), perfluorononanoic acid (PFNA) (0.067–0.152 ng/l), 2,4,5-T (0.046–0.27 ng/l), mecoprop (0.018–

0.24 ng/l), clofibric acid (<0.025–0.186 ng/l), bezafibrate (0.021–0.140 ng/l), gemfibrozil (0.024–0.180 ng/l), bentazone (0.068–0.150 ng/l), dichlorprop (<0.054–0.200 ng/l), irgarol (<0.007–0.131 ng/l), atrazine-desethyl (<36–86 pg/l), perfluorodecanoic acid (PFDA) (19 pg/l), isoproturon (<10–35 pg/l), and sulfadimethoxine (87 pg/l) (Table 1).

Comparison with other studies

It is important to perform a comparison of our monitoring data with other data from the literature in order to accurately interpret our results. There is relatively little information on the behavior and fate of pharmaceuticals or other anthropogenic compounds in fresh or marine waters. To our knowledge, there is only one study on the microbial degradation of pharmaceuticals in estuarine and coastal seawater [68]. Microbial degradation rates were measured in the New York City region for 19 pharmaceuticals in estuarine and coastal surface water samples. According to this study, antipyrine, carbamazepine, cotinine, sulfamethoxazole, and trimethoprim were the most refractory substances, with half-lives between 35 and > 100 days. Ying and Kookana [69] reported that the four endocrine-disrupting compounds—bisphenol A, 17-beta-estradiol, 17-alpha-ethynylestradiol, and 4-*t*-octylphenol—were largely degraded in coastal seawater within 56 days, but only after a lag time of several weeks.

Benzotriazoles

Our analytical results for the benzotriazoles (1*H*-benzotriazole: 3.0–9.2 ng/l; 5-methyl-1*H*-benzotriazole: 3.1–8.5 ng/l) are in relatively good agreement with recent analytical data from the North Sea, where Wolschke and coworkers [18] found up to 21 ng/l 1*H*-benzotriazole and up to 37 ng/l methylbenzotriazole in coastal areas. In the open North Sea, approximately 300 km distance from the coast, concentrations of benzotriazole and methylbenzotriazole (MBT) were 1.4 and 1.1 ng/l, respectively. These levels are very similar to the concentrations found in our study for the northern Adriatic Sea. However, it must be noted that we had only one MBT standard (5-MBT, not 4-MBT). Therefore, we cannot establish whether we detected both isomers or only one of them, considering that greater stability was reported for the 4-MBT isomer [70, 71]. However, [18] also used the 5-MBT standard.

Caffeine

In comparison to other studies, we detected relatively high concentration levels of caffeine (82–367 ng/l) in the northern Adriatic Sea. Weigel and coworkers [22, 23] reported lower concentrations of 2–16 ng/l in the open North Sea. Caffeine levels in coastal seawaters close to Stockholm (Sweden) were 30–74 ng/l [21], 7–87 ng/l close to Tromsø (Norway) [24], 5–71 ng/l in Massachusetts Bay seawater [72], and 5–149 ng/l on the west coast of Vancouver Island, British Columbia, Canada [73]. These results obtained for marine water samples indicate that the level of caffeine varies widely according to the location of the marine sampling site.

Caffeine is efficiently removed in well-functioning WWTPs (>99 %). Nevertheless, it is ubiquitously present in the aqueous environment, due to its extensive use [74]. It degrades relatively slowly by direct photolysis (>170 h in artificial sunlight), but enhanced photodegradation was observed in waters containing fulvic acids [75]. Work by Benotti and Brownawell [68] noted variable half-lives for caffeine (3.5 to >100 days) in estuarine and coastal marine samples.

Sucralose

Our results for sucralose can be compared to the study by Mead and coworkers [43], who presented the first findings of sucralose in coastal and open ocean waters (from the USA: the Florida Keys and the Gulf Stream), where they found sucralose concentrations of up to 392 ng/l in coastal waters and up to 67 ng/l in open sea water of the Gulf Stream. Our results clearly show lower sucralose levels for the Adriatic Sea (3.0–11.1 ng/l). This can be explained by lower sucralose use in Europe. In the USA, sucralose has been in use since 1998, whereas it was first authorized in 2004 in Europe.

Perfluoroalkyl substances (PFASs)

We found concentration levels of 1.3 ng/l PFOS and 1.3–2.5 ng/l PFOA in the northern Adriatic Sea. These levels are in good agreement with several other studies which showed that these compounds are entering the marine environment via rivers or atmospheric inputs of neutral precursor compounds, and can undergo long-range transportation via ocean currents [8, 39, 40, 42, 76]. In coastal and open waters of the North Sea, PFOS and PFOA concentrations of 0.25–4.2 ng/l and 0.11–7.8 ng/l were detected, respectively [38, 41, 51, 77]. PFBS levels of 1.50–4.13 ng/l were reported by Möller and coworkers [51] in the North Sea. Maximum levels found in the Atlantic Ocean were 73–291 pg/l and 229–439 pg/l for PFOS and PFOA [8, 39, 42, 76], and in Chinese (Pacific) coastal waters, concentrations of PFOS, PFBS, PFHpA, and PFOA were <21–70 pg/l, 23–941 pg/l, <11–422 pg/l, and 38–1,542 pg/l, respectively [40]. Recently, Sánchez-Avila and coworkers [78] published the first results for the Catalonian Coast of the Mediterranean Sea. They reported concentrations of <0.03–3.93 ng/l for PFOS and <0.08–1.86 ng/l for PFOA in coastal waters. Nearly all studies (except for [78]) suggest that levels of PFOA in marine waters are higher than PFOS concentrations.

It has been reported that short-chain polyfluoroalkyl substances (PFBS, PFBA) are replacing PFOS and PFOA in industrial processes and that PFBS is now the dominant PFAS in River Rhine surface water in Germany [51]. Unfortunately, we were not able to detect PFBS in the marine water samples due to an analytical problem with the detection of this compound. A huge double peak was detected at retention times of 3.46 min and 3.55 min for the PFBS transition m/z 299>80. However, the second transition (qualifier) m/z 299>99 had a retention time of only 3.56 min (Fig. S45 of the ESM), and the PFBS standard had a slightly different retention time of 3.61 min (Fig. S46 of the ESM). We concluded that there was an interference peak with the identical first PFBS transition, most likely originating from sulfophenyl carboxylates, a group of biodegradation intermediates of linear alkylbenzene sulfonates (LAS), a group of anionic surfactants.

Pesticides and pharmaceuticals

There is very little data on polar pesticides in marine water that allow a comparison of our results from the Adriatic Sea with those of other studies. Weigel and coworkers [22, 44] found similar concentrations for terbuthylazine (0.20–0.83 ng/l), metolachlor (0.25–0.61 ng/l), and desethylatrazine (1.6 ng/l) in the German Bight of the North Sea.

Reported concentrations of DEET, carbamazepine, clofibric acid, and mecoprop in the North Sea were <LOQ–1.09 ng/l, 2 ng/l [44], <LOQ–7.8 ng/l [25, 44], and 0.6–2.7 ng/l [25], respectively. In coastal waters close to Stockholm (Sweden), concentrations of carbamazepine, and metoprolol of 4–26 ng/l and 8–210 ng/l were found, respectively [21]. In seawater close to Tromsø (Norway), concentrations of DEET, and ibuprofen were 0.4–13 ng/l, and <LOQ–7.7 ng/l, respectively [24]. In estuarial seawater in Singapore, the pharmaceuticals naproxen, diclofenac, gemfibrozil, and ibuprofen were found at levels of 13–30 ng/l, 4–38 ng/l, 1–9 ng/l, and 41–121 ng/l, respectively [26]. These findings are also in relatively good accord with our results.

Conclusions

Most of the target chemicals analyzed were found for the first time in the surface waters of the northern Adriatic Sea. The chemicals detected were pesticides or their degradation products, pharmaceuticals, antibiotics, benzotriazoles, sucralose, and PFASs. Very low LOQs (in the picogram per liter concentration range) were achieved by extracting only 1 l of water. The multi-compound analysis method developed here is an important tool for implementing the Water Framework Directive (WFD) and Marine Strategy Framework Directive (MSFD). The WFD requires compliance monitoring of priority pollutants against the environmental quality standards (EQS) in coastal waters; in addition, biota analysis of fish and shellfish will become more important. Trend analysis in open marine waters is necessary for the MSFD to ensure that there are no upward trends for persistent pollutants in the environment. More temporal and spatial trend analysis of the marine environment will be necessary in the future. Chemical pollution of the environment is one of the global boundaries that must not be transgressed, in order to prevent unacceptable environmental changes due to human activities. Crossing certain biophysical or chemical thresholds could have disastrous consequences for humanity [79].

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