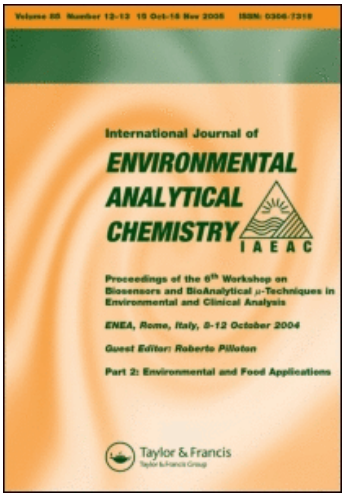

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Determination of monohydroxylated metabolites of polycyclic aromatic hydrocarbons (OH-PAHs) from wastewater-treatment plants

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A high-performance liquid chromatography separation coupled with mass spectrometry via an electrospray interface is proposed for the determination of the hydroxylated derivatives of polycyclic aromatic hydrocarbons (OH-PAHs) in treated and untreated wastewaters and suspended solids from sewage treatment plants (STPs). The developed SPE procedure was applied to spiked wastewater samples, with recovery yields (1000 mL; 100 ng L^{-1} spiking level) in the 65–87% (RSD: 6–12%) range for the selected OH-PAHs. The limits of detections ranged between 0.3 and 3.2 ng L^{-1}, depending on the selected compound and on the investigated matrix. The proposed method was applied to the determination of the selected analytes in real samples from a sewage-treatment plant (STP). The investigated OH-PAHs were detected mainly in the particulate fraction. The exhibited mean concentrations of positive samples (as the sum of dissolved and particulate matter) in the STP final effluent ranged from 15 to 68 ng L^{-1}.

Keywords: Hydroxylated PAHs; LC-MS; Solid-phase extraction; Sewage-treatment plant

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants included in both US-EPA and EU priority pollutant lists, as well as in international guidelines for waste-monitoring programmes [1, 2]. PAHs are moreover known to be transformed by microbiological and/or photochemical degradation into aromatic derivatives such as nitro-PAHs, halo-PAHs, oxy-PAHs, and hydroxy-PAHs, some of which are suspected of posing an additional hazard to humans and living organisms due to mutagenic and carcinogenic effects [3, 4]. Moreover, some mono-hydroxy derivatives, such as 3-hydroxybenzo[a]pyrene, as well as their parent PAHs, have been recently shown to exhibit oestrogenic and anti-oestrogenic effects [5, 6]. The hydroxylated derivatives (OH-PAHs) have been investigated mainly in biological samples, such as plasma, urine, and bile, since they are distinctive biomarkers for

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assessing the human exposure to PAHs. 1-OH-Pyrene and 1-OH-phenanthrene, the urinary metabolites of pyrene and phenanthrene, respectively, are commonly used to trace the exposure to PAHs. The analytical methods proposed so far for the determination of OH-PAHs are mainly based on liquid chromatography coupled with fluorescence [7], electrochemical [8] or mass spectrometry [9–11] detection, or on gas chromatography coupled with mass spectrometry [12, 13]. Micellar electrokinetic capillary chromatography has also been proposed for their separation [14]. In some cases, enzymatic hydrolysis of their conjugated metabolites (e.g. glucuronides and sulfates) after solid-phase extraction (SPE) was applied to biological samples [7, 10]. Since typical concentration levels of OH-PAHs in biological matrices are in the ppb–ppm level, processed volumes usually do not exceed 50 mL. Moreover, some OH-PAHs have been recently analysed also in cigarette-smoke condensate [15].

Nevertheless, very little information is available, to the best of our knowledge, on the presence, distribution, and behaviour of OH-PAHs in the environment. The available data refer generally to OH-PAHs in tissues and fluids collected from aquatic and terrestrial organisms, to be used as biomarkers for PAH contamination [7, 16, 17]. It is known that biological degradation processes by microorganisms similar to those existing in humans and animals could occur also during sewage treatment or in surface waters. In addition, the non-biological formation of OH-PAHs was also recorded as result of photochemical or oxidation reactions. Stir-bar sorptive extraction (SBSE) coupled with chromatography coupled with mass spectrometry (GC–MS) has been recently proposed for sea water and puddle water, while high-performance liquid chromatography (HPLC) coupled with fluorescence detection has been used to ascertain the presence of OH-PAHs in airborne particulate matter [12, 18].

The aim of this study was to develop an analytical method based on SPE followed by liquid chromatography coupled with mass spectrometry detection (SPE–HPLC–MS) for the determination of selected OH-PAHs in sewage-treatment plants (STPs). The proposed analytical method was applied to the preliminary determination of the named analytes in a municipal-industrial STP in order to evaluate their occurrence and distribution, and to evaluate the formation of hydroxylated metabolites from PAHs entering the STP, which were previously identified in the same plant [19]. The selected analytes were those containing two to four condensed rings, obtained by hydroxylation of the most frequently recorded parent PAHs in wastewaters.

2. Experimental

2.1 *Materials and methods*

The examined hydroxylated PAHs (1-naphtol, 2-naphtol, 1-hydroxy phenanthrene, 1-OH-PHEN, 10 ng μL^{-1}, purity 99.0%; 1-hydroxy pyrene, 1-OH-PYR, 10 ng μL^{-1}, purity 99.9%; 6-hydroxy chrysene, 6-OH-CHRY, 10 ng μL^{-1}, purity 99.9%) were purchased from Dr Ehrenstorfer (Augsburg, Germany). The tested sorbents were Discovery DSC-18, Supelclean ENVI-18 and Envichrom-P from Supelco (Bellefonte, PA), and Strata-E from Phenomenex (Torrance, CA). The glass-fibre filters (GF/F, 0.7 μm) were purchased from Whatman (Clifton, NJ). Acetonitrile (ACN), methanol (MeOH), 2-propanol, acetone, tetrahydrofuran (THF), dichloromethane (DCM),

and *n*-hexane were HPLC ultra-gradient solvents from Romil (Dublin). Water for chromatographic purposes was purified using a Milli-Q system (Millipore, Bedford, MA). All working standard solutions were prepared daily by diluting the PAH stock solutions in 2-propanol with an Agilent G1313A autosampler (Avondale, PA). Standard solutions, working solutions, and sample extracts were stored in brown glass vials (Agilent) at 2°C in order to prevent photochemical degradation. All laboratory materials for analytical purposes were accurately cleaned with ammonium persulfate solution, and then rinsed twice with hexane and 2-propanol before use. The GF/F glass-fibre filters were pre-cleaned by sonication (2 h) with 2-propanol and then gently dried overnight (12 h at 80°C). Because of their potential hazard, all standards and application samples were handled with appropriate safety precautions, as suggested by Occupational Safety and Health Administration (OSHA) guidelines [20].

2.2 *Hydroxy-PAHs extraction from water samples*

Hydroxylated PAHs (OH-PAHs) were extracted (triplicate determination plus a procedural 'blank') from 1000 mL of water (MilliQ, wastewater, STP final effluent) samples by SPE on Strata-E cartridges (1 g, 6 mL) with an automated SPE-system Aspec XL by Gilson (Middleton, WI). Water samples were acidified at pH 2.5 by HCl (37% in water, v : v) and MeOH was added (3%, v : v) to each sample prior to extraction. The sorbent phase was conditioned at 5 mL min^{-1} by sequential elution of 9 mL of MeOH and 12 mL of a water : MeOH (97 : 3, v : v) solution acidified at pH 2.5 with HCl (37% in water). Water samples were passed through the cartridges at 10 mL min^{-1}. The cartridges were then washed with 30 mL of a water : MeOH 90 : 10 (v : v) solution acidified at pH 2.5 and then vacuum-dried for 1 h by means of a SPE Manifold system (Supelco). The elution was then performed by passing through the cartridges 1 mL of MeOH followed by 10 mL (5 mL × 2) of a DCM : THF 90 : 10 (v : v) solution at 1 mL min^{-1}. Two mL of 2-propanol were added to the extracts, which were then concentrated to 500 μL under a gentle nitrogen flow in a Turbovap II automated evaporator by Zymark (Hopkinton, MA). The final extracts were then diluted to 1 mL with 2-propanol and stored in 2-mL Teflon capped screw-cap glass vials at 2°C before injection (50 μL) in the chromatographic system.

2.3 *Hydroxy PAHs from STP suspended particulate matter*

Real suspended particulate matter (SPM) samples retained by the 0.7 μm filters from 0.5 l of raw sewage and 3 L of final effluent, respectively, were used as reference matrix. Hydroxy-PAHs were extracted from lyophilized filters by sonication using a DCM : THF (90 : 10, v : v, 15 mL × 3, 1 h each aliquot solvent) mixture. Extracts were then filtered at 0.7 μm and concentrated down to 500 μL in the automated evaporator. No further clean-up procedures were applied. The final extracts were then diluted to 1 mL (enrichment factor: 1000) with 2-propanol and stored in 2-mL Teflon capped screw-cap glass vials from Agilent at 2°C before their injection (50 μL) in the chromatographic system.

2.4 *Hydroxy-PAHs chromatographic separation and detection*

The extracts were injected in an Agilent (Palo Alto, CA) HPLC 1100 system using an Agilent G1313A autosampler. The analytes were separated by an Envirosep-PP (Phenomenex), C-18, 150×4.6 mm, 5 μm, protected by two C18, 4×3 mm guard columns (Phenomenex). Two guard columns were found to be highly recommendable, in comparison with one, for extracts such those investigated in order to carry out long analysis sequences without any noticeable degradation of the chromatographic performances. The LC column was kept at 15°C by an Agilent G1316A thermostated column compartment. Acetonitrile (A) and Milli-Q water (B) were used as the mobile phase at a flow rate of 0.6 mL min^{-1}. The gradient elution programme was 20% A, holding for 2 min and then increasing to 99% A in 30 min.

The detection of OH-PAHs was performed using an Agilent 1100 MS Quadrupole detector via an electrospray interface (ESI) operating under negative ionization (NI) conditions. Nebulizing and drying gases were nitrogen kept at 40 psi and 350°C, 10 L min^{-1}, respectively. The capillary and fragmentator voltages were set at 5500 V and 125 V, respectively. The analytes were identified by both retention time and compound mass/charge ratio (m/z). The quantification of OH-PAHs in the real sample extracts was performed by the standard addition method (five-point calibration curve). An $R^2 > 0.994$ linearity was observed in the 1–100 ng (as injected amount) range. The operation and settings of the MS detector were controlled by an Agilent 1100 Chemstation LC/MSD (Ver. 8.03) software. The limits of detection (LODs) were calculated in real sample extracts as the minimum analyte concentrations which could generate a signal-to-noise (S/N) ratio of 3. The determined values are reported in table 1.

2.5 *Sampling and sample pre-treatment*

Average 24-h samples (municipal wastewaters, industrial wastewaters, treated effluents) were collected by means of autosamplers on September 2003 at the mechanical–biological sewage treatment plant (STP) of Fusina (Venice, Italy). The samples were then acidified at pH 2.5 with HCl (37% in water) and stored in the dark at 2°C in dark glass bottles prior to extraction to avoid photochemical degradation. The sampling sites were named as follows: Site A, municipal wastewater inlet; Site B, industrial wastewater inlet; Site C, treated effluent.

Ten millilitres of an $HgCl_2$ (100 mg L^{-1} in water) solution was added to the samples just after collection in order to prevent bacterial degradation. The suspended particulate matter (SPM) was obtained by filtration of collected water samples on 0.7 μm

Table 1. Monitored ions, retention times, and determined LODs for the examined hydroxylated PAHs.

Analyte	m/z $[M-H]^-$	Retention time (min)	LOD, MilliQ water (ng L^{-1})	LOD, STP effluent (ng L^{-1})	LOD, STP influent (ng L^{-1})
1-OH-NAP	143.0	6.1	0.4	1.8	3.2
2-OH-NAP	143.0	6.5	0.4	1.5	3.2
1-OH-PHE	193.1	9.8	0.2	1.0	1.8
1-OH-PYR	217.1	12.4	0.1	0.6	1.5
6-OH-CHR	243.1	13.6	0.05	0.3	0.9

glass-fibre filters. Filters were then lyophilized at −60°C and stored in the dark in aluminium foil at 2°C prior to extraction. All chemical analyses were performed within 96 h of sampling.

3. Results and discussion

3.1 *Extraction of hydroxylated PAHs from water samples*

To the best of our knowledge, no analytical methods have been previously reported for the determination of OH-PAHs in wastewater samples. A new extraction procedure was first developed. The SPE technique was chosen for the extraction, enrichment, and clean-up of OH-PAHs in waste-water samples for its versatility [21, 22]. Since typical SPE parameters such as sorbents, flow rates through SPE cartridges, type and content of the organic modifier, breakthrough volumes, eluting solvents, matrix effects, and evaporation losses are well known to affect the recovery rates and the method reproducibility for the parent PAHs, several preliminary experiments were carried out to optimize the various steps, on the basis of results previously found for PAHs [19]. These preliminary experiments were carried out by spiking 1000 mL of Milli-Q water (triplicate determination, plus a procedural 'blank') with a solution containing the five selected OH-PAHs (1-hydroxy-naphtalene, 2-hydroxy-naphtalene, 1-hydroxy-phenanthrene, 1-hydroxy-pyrene, 6-hydroxy-chrisene) at the final concentration of 100 ng L^{-1} for each compound (table 2). Various sorbent phases were tested: Discovery DSC18, Supelclean Envi-18 and EnviChrom-P from Supelco, and Strata-E from Phenomenex. Since similar log K_{ow} values were exhibited by OH-PAHs, in comparison with their parent PAHs (3.9–5.3 *vs.* 4.4–5.5) (as calculated by KOWWIN© software when no experimental values were available in the open literature [23]), the use of typical solvents used for PAHs elution, such as 2-propanol, acetone, hexane, was assumed to be effective for the elution and clean-up step of OH-PAHs from C-18 SPE cartridges.

However, this was not the case: the use of 2-propanol, acetone, or hexane as eluting solvents yielded very low (<30%) recoveries. Similar recovery efficiencies were also

Table 2. Determined recoveries ($n = 3$) and correspondent relative standard deviations (RSDs,%) obtained for the examined hydroxylated PAHs with different sorbing phases and eluting solutions (Sol A: DCM; Sol B: 1 mL MeOH + 10 mL DCM : THF 90 : 10).[a]

	Sol A								Sol B	
	DSC-18		Envi-18		Envi-Chrom P		Strata-E		Strata-E	
OH-PAH	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)
1-OH-NAP	89	7	92	6	90	6	95	7	97	7
2-OH-NAP	91	8	88	6	87	8	94	7	96	6
1-OH PHE	92	8	87	7	83	8	92	7	94	6
1-OH PYR	61	8	44	7	45	7	57	7	92	6
6-OH CHR	44	7	37	7	33	5	50	7	97	5

[a]Processed volume: 1000 mL of MilliQ water. Spiked concentration: 100 ng L^{-1} each analyte.

found when 10 mL of *n*-hexane : acetone : 2-propanol 90 : 5 : 5 mixture was used, according to an extraction procedure previously developed for PAHs [19]. Higher (50–92%) but highly fluctuating recovery values were instead observed when pure dichloromethane (DCM) (Sol A) was used as eluting solvent. Satisfactory recoveries were finally obtained by eluting the selected analytes with 1 mL of methanol followed by 10 mL of a DCM : THF (90 : 10, v : v) solution (Sol B). The recovery efficiencies found with the tested sorbing phases and using Sol A and Sol B as eluting solutions are reported in table 2. The Strata-E was found to be the best sorbent phase among those tested, offering very satisfactory recovery rates (92–97%, RSD: 4–6%). The extraction step with Sol B was afterwards developed only on strata E stationary phase, since it exhibited a better performance. The initial elution with 1 mL MeOH seemed to be very important in eliminating water residues from the sorbent phase before the subsequent elution step, since recovery yields without this step were found to be much lower (64–87%) and with higher (12–17%) RSDs.

On the basis of this experimental evidence, the presence of a hydroxylic group on a multiaromatic structure seems to induce an unexpected retention behaviour for the molecule. As previously found for PAHs, only a solvent mixture containing a major apolar solvent fraction and a minor polar fraction seemed to elute aromatic analytes efficiently from the sorbent phase. The exhibited recoveries were higher than those reported previously by SBSE, and similar to those found with C18 cartridges, where a much lower volume (5 mL of MilliQ water spiked at 500 $\mu g\,L^{-1}$) was processed [11, 12].

The developed SPE procedure was extended also to spiked real aqueous samples from a sewage-treatment plant (STP), such as raw sewage and STP final effluents. The results are reported in table 3. The spiked (1000 mL processed sample, 100 $ng\,L^{-1}$) municipal raw wastewater showed average percentage recoveries for OH-PAHs in the 65–81% range (RSD: 6–11%), while the treated effluents showed average percentage recoveries in the 80–87% (RSD: 6–12%) range, respectively. By comparing the results reported in tables 2 and 3, it follows that the overall matrix effects in the STP water samples resulted, as previously found for their parent PAHs, in a 10–32% recovery diminution, in comparison with spiked Milli-Q water surrogate samples [19].

3.2 *HPLC-MS detection for hydroxylated PAHs*

The hydroxylated PAHs are known from the literature to exhibit intense signal responses using an electrospray ionization interface (ESI) in the negative (NI)

Table 3. Determined recoveries and relative standard deviations (RSDs, %) obtained for the examined hydroxylated PAHs on Strata-E sorbing phase and eluting solution B.[a]

	Municipal influent		Industrial influent		Final effluent	
Analyte	Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
1-OH-NAP	81	11	76	8	77	11
2-OH-NAP	78	9	78	9	79	12
1-OH-PHE	75	10	80	6	84	9
1-OH-PYR	69	8	82	6	80	8
6-OH-CHR	65	6	87	7	87	6

[a]Processed volume: 1000 mL of raw municipal wastewater, partially treated industrial wastewater, and treated effluent. Spiked concentration: 100 $ng\,L^{-1}$ each analyte.

ionization mode, with the formation of $[M-H]^-$ pseudo-molecular ions [10], while their parent PAHs show a very low ionization efficiency under the same detection conditions [24]. MS parameters (fragmentator and capillary voltage, drying gas flow, nebulizer pressure, and drying gas temperature) were optimized by flow injection analysis (FIA) experiments, in order to achieve the best S/N ratio suitable for their subsequent detection in environmental samples. Under the adopted detection conditions, the area linearity of the MS detector was found to be acceptable for all investigated compounds in the 1–100 ng interval (as injected amount), showing a 0.9939–0.9998 (as R^2) linearity range (average: 0.9981). The area reproducibility (obtained from ten consecutive injections of 1 μL of a 10 ng μL^{-1} standard mixture solution) was in the 2.6–10% (as RSD) interval. The limits of detection (LODs, S/N = 3) in spiked MilliQ water resulted in the 0.05–0.4 ng L^{-1} range (table 1). The exhibited LODs in real matrices were higher, as expected due to the matrix effects: 0.9–3.2 ng L^{-1} and 0.3–1.8 ng L^{-1} in raw sewage and STP final effluent, respectively. These values are similar to the method recently proposed by SBSE-GC-MS [12].

3.3 *Determination of hydroxy-PAHs in real samples*

The proposed procedures were applied to the determination of the selected OH-PAHs in aqueous and solid samples collected at the mechanical/biological STP of Fusina (Venice, Italy). This STP daily receives approx. 98,000 m^3 of municipal wastewaters (corresponding to approx. 300,000 equivalent inhabitants) from mainland residential districts and partially treated industrial discharges from the Porto Marghera industrial area (approx. 30,000 equivalent inhabitants). In addition, approximately 300 m^3 of untreated industrial sewage is daily treated by the STP. The final effluent is then discharged in the Venice lagoon, a fragile coastal ecosystem undergoing several anthropogenic pressures [25].

The hydroxylated PAHs were investigated in the same matrices where PAHs were previously identified in order to evaluate their presence, formation, and distribution during sewage treatment [19]. A screening investigation was carried out in order to infer the presence and distribution of the selected analytes in some representative sampling sites in the investigated STP. Two 24-h samples were averaged before analysis. The analysed samples can be considered as representative, since the dimension of the plant and of its sewage network (table 4). The investigation was limited to the three- and four-ring OH-PAHs, since they could arise from the most commonly occurring PAHs, while the two ring analytes (i.e. 1-OH-NAP and 2-OH-NAP), even if selected for

Table 4. Concentrations (filtered, filtered + suspended) of the selected OH-PAHs in the different stages (raw municipal wastewater, partially treated industrial wastewater, treated effluent, 24-h averaged samples) of the examined STP.

Analyte	Mun. influent, (filtered phase) (ng L^{-1})	Mun. influent, (ng L^{-1})	Ind. Influent, filtered phase (ng L^{-1})	Ind. Influent (ng L^{-1})	Final effluent, filtered phase (ng L^{-1})	Final effluent, (ng L^{-1})
1-OH-PHE	< LOD	< LOD	< LOD	32 ± 5	< LOD	8 ± 1
1-OH-PYR	< LOD	87 ± 7	459 ± 22	521 ± 26	< LOD	12 ± 1
6-OH-CHR	< LOD	23 ± 2	848 ± 43	864 ± 44	< LOD	< LOD

testing the extraction step efficiency, were not investigated in real samples, since they could arise from other industrial sources, such as impurities or additives. The selected OH-PAHs were detected in approximately half of the analysed samples, especially in the industrial sewage: the recorded total concentrations (filtered + suspended) ranged between 44 and 342 ng L^{-1} in the municipal influent, and between 293 and 477 ng L^{-1} in the industrial influent, respectively. Both 1-OH-pyrene (1-OH-PYR) and 1-OH-chrysene (1-OH-CHR) were found in most analysed samples. The selected analytes in the final effluent were recorded mainly in the particulate phase, with mean concentrations (filtered + suspended) in the 15–68 ng L^{-1} range, with approximately half exhibiting < LOD values. A comparison with the found concentration for their parent PAHs in the same samples, determined in the same samples by applying an analytical method recently reported [19], indicates that their hydroxylated metabolites are surprisingly more important in the examined industrial influent, in comparison with the municipal influent: the recorded concentrations of OH-PAHs were 11–21% of their corresponding parent compounds in the municipal sewage, while they were up to 6.4 times the total PAHs concentration in the industrial influent, thus indicating that not only biological degradation but also chemical oxidation during physico-chemical pretreatment of industrial sewage could be a significant source of hydroxylated PAHs in the environment. The selected OH-PAHs in the final effluent showed the same ratios as in the municipal influent: 9, 21, and 26% of their parent PAHs for 1-OH-PHE, 1-OH-PYR, and 6-OH-CHR, respectively. The suspended solids were moreover shown to contribute most to the overall input of OH-PAHs in the Venice lagoon, as for their parent compounds, since the concentration values in the dissolved phase were always < LOD (table 4). This result is not surprising, on the basis of the behaviour of hydroxy-PAHs, as revealed during the extraction procedure development: the analysed chemicals (1-OH-PHE, 1-OH-PYR and 6-OH-CHR) exhibit log K_{ow} values of 3.9, 4.5, and 5.30 (calculated by KOWWIN© software), only 0.49–0.22 units lower than those exhibited by their parent compounds (4.4, 4.9, and 5.5, respectively, as calculated by the same software [23]). On the basis of this experimental evidence, the absorption to the sludge seems the main elimination pathway of these PAH derivatives, as for their parent compounds. Figure 1 shows a typical HPLC-MS chromatogram of an STP effluent extract, showing the Full-Scan MS Total Ion Current (TIC) in the 180–300 m/z range, and the signals corresponding to the investigated 1-OH-PHE, 1-OH-PYR, and 6-OH-CHR (the bold areas are those assigned by both retention times and m/z values to the investigated isomers). It is noteworthy that other peaks of similar intensity, which could be attributed to other OH-PAHs isomers because of similar retention times and the same m/z ratios, were also detected, but they could not be quantified in the absence of confirmatory reference standards. Phenanthrene, pyrene, and chrysene can in fact theoretically produce up to five, five, and six hydroxylated isomers, respectively. As shown in the chromatograms, the other detected peaks can account (by assuming the same response factors for all isomers) up to four to six times the recorded values, so the actual overall concentrations of hydroxylated PAH leaving the STP could be strongly underestimated by taking into account only the selected isomers. Other OH-PAH isomers should be available as reference standards in order to ascertain the reported hypothesis.

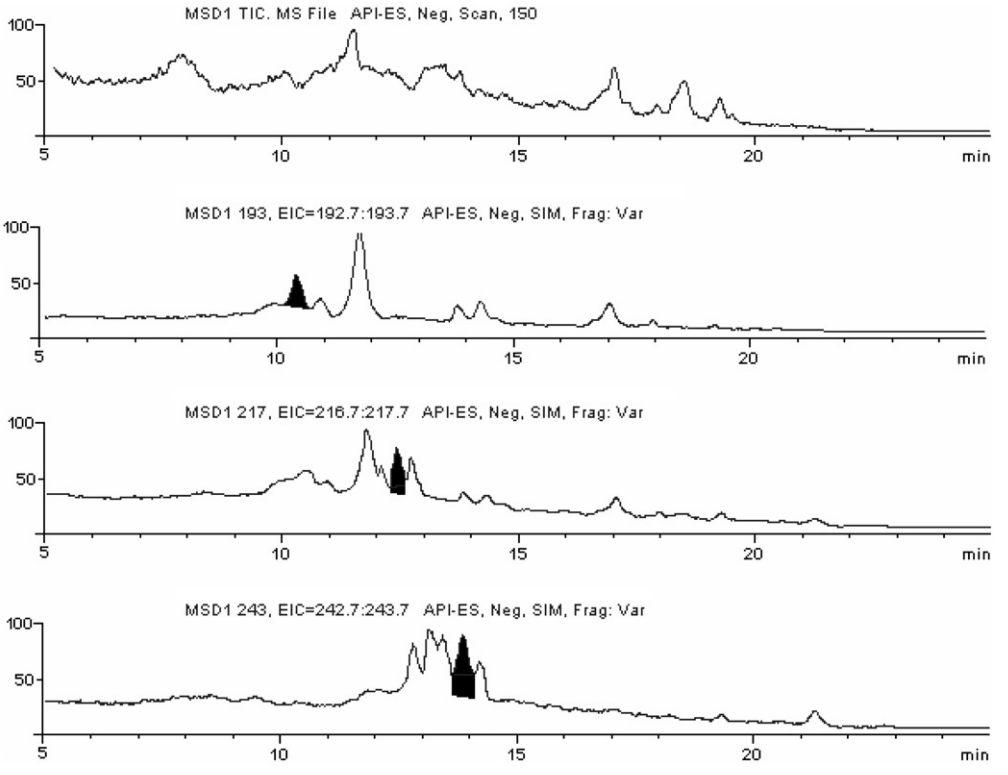

Figure 1. Typical HPLC–ESI–MS chromatogram of an SPE extract from a final effluent (suspended phase) sample collected in the investigated STP. Upper trace: total ion current (TIC) in the 200–300 m/z range; lower traces: single ion monitoring (SIM) traces corresponding to 193, 217, and 243 m/z values.

4. Conclusions

An analytical method based on SPE–HPLC–MS for the determination of hydroxylated PAHs in treated and untreated STP wastewaters has been proposed. The developed method allowed the determination of OH-PAHs in both dissolved and suspended phase of aqueous samples from STPs. Despite the limited number of selected compounds, the method was found to be sufficiently robust and reliable for the determination of hydroxy-PAHs in such complex matrices. Further work is in progress to determine other OH-PAHs in the same matrices in order to evaluate their overall contribution to the oestrogenic potential of sewage effluents and receiving surface waters in the Venice lagoon.

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