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Determination of endocrine-disrupting phenols, acidic pharmaceuticals, and personal-care products in sewage by solid-phase extraction and gas chromatography–mass spectrometry

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Abstract

The occurrence, fate, and effects of phenols with endocrine-disrupting properties as well as some pharmaceuticals and personal-care products in the environment have frequently been discussed in recent literature. In many cases, these compounds were determined by using individual methods which can be time-consuming if results for multiple parameters are required. Using a solid-phase extraction procedure with an anion exchanger in this work, we have developed and optimized a multi-residue method for the extraction of 21 phenols and acids in sewage influent and effluent. The phenols and acids were then selectively eluted in separate fractions and were converted into pentafluoropropionyl (PFP) and *tert*-butyldimethylsilyl (TBDMS) derivatives, respectively, for gas chromatography–mass spectrometric (GC/MS) determination. When applied to the sewage samples under study, the results for nonylphenol, bisphenol A (BPA), triclosan (TCS), 17ß-estradiol (E_2), estrone (E_1), salicylic acid, ibuprofen, naproxen, diclofenac, and a few other acidic drugs were consistent with those determined previously by individual methods. Using the same procedure, we also report, for the first time, the occurrence of 2-phenylphenol and parabens in those sewage samples.

Keywords: Endocrine-disrupting chemicals; Phenols; Pharmaceuticals; Personal-care products; Sewage

1. Introduction

In the last two decades, a great deal of research effort has been devoted to the identification, occurrence, fate, and effects of organic contaminants found in municipal wastewaters [1,2]. Among these organics, chemicals that have the potential to disrupt the normal functions of the endocrine system in wildlife, fish, and humans have drawn special attention [3]. Collectively known as endocrine-disrupting chemicals (EDCs), they encompass a wide range of industrial and household chemicals such as polychlorinated biphenyls (PCBs), chlorinated insecticides, alkylphenols and their ethoxylates, bisphenol A (BPA), etc. Pharmaceuticals used for humans, farm animals, and fish have also been identified as emerging environmental pollutants [4]. Many classes of common prescription and non-prescription drugs, including analgesics, anti-inflammatories, antiepileptics, ß-blockers, antibiotics, etc., are discharged into sewage systems at the end of their application-cycles. The environmental concentrations of many pharmaceuticals and personal-care products (PPCPs) have also been documented in detail [4–6].

The occurrence of EDCs as well as PPCPs in municipal sewage effluent and other environmental samples could negatively impact the health of the ecosystem and humans. The environmental occurrence of EDCs has been implicated in the feminization of fish, sex transposition of wildlife, and hormone-related cancers in humans [7]. The detection of carbamazepine, a commonly prescribed antiepileptic, in influent and effluent samples at nearly the same concentrations exem-

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plifies that a persistent drug could survive existing wastewater treatments [8]. As carbamazepine and other pharmaceuticals are biologically active compounds, their presence in aquatic ecosystems presents a potential hazard to human health if they contaminate our drinking water. A recent study suggests that wide-spread veterinary use of diclofenac, an anti-inflammatory drug, is indirectly causing the near extinction of vultures in South Asia [9]. Hence, there is a need to monitor the levels of EDCs and PPCPs in sewage and other environmental samples.

In response to the specific needs of different research projects in the past, analytical methods have been developed separately for EDCs such as 4-nonylphenol (NP) and *tert*-octylphenol (OP) [10], BPA [11], 17ß-estradiol (E_2) and its metabolites [12], as well as those for acidic pharmaceuticals [13]. Generic adsorbents such as the reversed-phase octadecylsilane (ODS), the Oasis HLB, and others have been applied by many workers to the extraction of the above EDCs and PPCPs in water and wastewater samples with good success [14–18]. In those cases, however, phenols and acids were eluted from the cartridge by methanol in the same fraction. For a complex matrix such as sewage influent, the non-selective ODS/HLB enrichment procedures occasionally yielded an extract with excessive coextractives. Such coextractives would lead to the formation of emulsion in the back extraction steps, reduced yields in chemical derivatization, and interference in GC/MS analysis. Recently, cartridges packed with anion exchangers have also been successfully applied to the extraction of acids and phenols in the environment [19]. In this work, we shall describe an optimized method for the extraction of 21 phenols and acids in sewage samples using an anion exchanger, with a subsequent elution procedure to fractionate the phenols and acids. This multi-residue procedure produces a cleaner extract and minimizes sample preparation time in screening applications.

2. Experimental

2.1. Chemicals and reagents

All standards including the phenols listed in Table 1 (the parabens, 2-phenylphenol, the alkylphenols, BPA, BPA-d_{16}, triclosan (TCS, 5-chloro-2-(2,4-dichlorophenoxy)phenol), estrone (E_1), E_2, E_2-d_3, the acidic pharmaceuticals listed in Table 2, as well as 2,3-dichlorophenoxyacetic acid (2,3-D, a recovery standard used for the acidic pharmaceuticals) were products of Sigma–Aldrich Chemicals. The derivatization reagents, pentafluoropropionic acid anhydride (PFPA) and *N*-*t*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) with 1% TBDMSCI, were also purchased from the same company. 4-*n*-Nonylphenol (nNP) was a Pestanal standard available from Riedel-deHaën (through Supelco Canada).

Table 1
Phenols included in this study and their characteristic ions (as PFP derivatives)

Phenol	Retention time (min)	Molecular ion, *m*/*z*	Characteristic ions, *m*/*z*
Methyl paraben, MP	5.51	298	298, 267
Ethyl paraben, EP	6.10	312	312, 284, 267
Propyl paraben, PP	7.06	326	285, 284, 267
Butyl paraben, BP	8.27	340	285, 284, 267
2-Phenylphenol, PHP	7.09	316	316, 197, 169
4-*tert*-Octylphenol, OP	7.76	352	352, 281, 253
4-Nonylphenol, NP	8.7–9.9	366	366, 309, 295, 281, 267, 253
4-*n*-Nonylphenol, nNP	11.71	366	366, 253, 254
Bisphenol A, BPA	13.52	520	520, 506, 505
Bisphenol A-d_{16}	13.43	534	534, 517, 516
Triclosan, TCS	14.19	434	434, 254, 252
Estrone, E_1	16.10[a]	416	416, 372, 359
17ß-Estradiol, E_2	14.84[a]	564	564, 401
17ß-Estradiol-d_3, E_2-d_3	14.81[a]	567	567, 404

Underlined ions were used for quantitation.
[a] A different temperature program was used for the estrogens.

2.2. Sample collection and storage

From May to July 2004, 24 h composite influent and effluent samples were collected from eight sewage treatment plants located in Burlington, Galt, Guelph, Mississauga, Toronto (three plants), and Waterloo in southern Ontario, Canada. These cities have populations ranging from less than 100,000 to over 1,000,000. All samples were mixtures of residential and industrial wastewaters in various proportions. They were collected by plant staff, transported immediately to our laboratory, and extracted upon arrival. If same-day extraction could not be achieved, the samples were stored at 4 °C in the dark and extracted within 48 h of their arrival.

Table 2
Acidic pharmaceuticals included in this study and their characteristic ions (as TBDMS derivatives)

Acidic pharmaceuticals	Retention time (min)	Molecular ion, *m*/*z*	Characteristic ions, *m*/*z*
Clofibric acid, CFA	9.15	328	273, 271, 143
Ibuprofen, IBP	9.51	320	264, 263
Salicylic acid, SYL	10.65	366	351, 310, 309
Gemfibrozil, GFB	13.70	364	307, 244, 243
Fenoprofen, FNP	14.36	356	300, 299
Naproxen, NPX	15.67	344	288, 287, 185
Triclosan, TCS	16.41	402	347, 345, 200
Ketoprofen, KTP	17.61	368	312, 311, 295
Tolfenamic acid, TFN	18.75	375	320, 318, 244
Diclofenac-Na, DCF	19.06	409	354, 352, 214
Indomethacin, IDM	25.46	471	414, 370, 139
2,3-D (surrogate)	11.56	334	279, 277, 249

Underlined ions were used for quantitation.

2.3. Solid-phase extraction (SPE) of samples

Immediately before extraction, a sewage sample was filtered through a 90 mm GF/C filter (1.2 μm retention) with a fritted, all-glass filtration device under a slight vacuum. Celite was added as a filter aid, particularly for the influent, to prevent clogging. To each litre of the filtered sample, 2.5 μg of 2,3-D, 0.5 μg each of nNP and BPA-d_{16}, and 0.05 μg E_2-d_3 were spiked as recovery standards. The sample was acidified to ca. pH 3 with sufficient HCl. Meanwhile, for each sample, a 6 mL, 150 mg Oasis MAX SPE cartridge (Waters Corp.) was conditioned by washing with 4 mL of methanol followed by 10 mL of water at pH 3. With a solid-phase extraction manifold (Supelco Visiprep 57044 or equivalent), the sample was siphoned through the cartridge at a flow rate between 10 and 15 mL/min by adjusting the vacuum. When the extraction was complete, the cartridge was dried for 1 min. The cartridge was then washed with 5 mL of a methanol/sodium acetate solution prepared by dissolving 0.41 g of anhydrous sodium acetate (NaOAc) in 5 mL of methanol and made up to 100 mL with water. The washing was discarded. This was followed by sequential elution with 5 mL of methanol (phenol fraction) and 10 mL of 2% formic acid in methanol (acidic pharmaceutical fraction). Both fractions were collected for derivatization.

2.4. Derivatization of phenols and acidic pharmaceuticals

To the phenol fraction, 25 μL of a methanolic solution of NaOAc (0.25 g of anhydrous NaOAc in 25 mL methanol) were added. It was evaporated to ca. 500 μL in a 40 °C bath using nitrogen. Then 1 mL of methyl *tert*-butylether was added and the volume was further reduced to 100 μL but not to dryness. This procedure was repeated two more times. The pentafluoropropionyl (PFP) derivatives of phenols were formed by the reaction of the extract with 100 μL of PFPA at room temperature for 20 min according to an earlier procedure [11]. The products were made up to a final volume of 1 mL in *iso*-octane for the analysis of phenols by gas chromatography–mass spectrometry (GC/MS). If an analysis of the estrogens was needed, an aliquot of the above methanol extract was evaporated to dryness and reacted with PFPA [12]. The products were washed by a base, back extracted by petroleum ether, evaporated to dryness, and redissolved in 250 μL of *iso*-octane for a separate GC/MS analysis of E_2 and E_1.

After the addition of 250 μL of 2% NH_4OH in methanol to the acidic pharmaceutical fraction, the solvent was evaporated to dryness, again in a 40 °C bath using nitrogen. To the residue, 50 μL of acetonitrile and 75 μL of MTBSTFA with 1% TBDMSCI were added. The mixture was then reacted at 75 °C for 30 min. At the completion of the reaction, the TBDMS derivatives were washed with water and back extracted into 3 mL of hexane and then 3 and 2 mL of 10/90 dichloromethane/hexane (v/v). The products were made up to a final volume of 1 mL in *iso*-octane for the GC/MS analysis of the acidic pharmaceuticals.

2.5. GC/MS analysis of phenols and acidic pharmaceuticals

A system consisting of an Agilent Model 6890 gas chromatograph, Model 7683 autosampler, and Model 5973 Mass Selective Detector was used for the GC/MS analysis in this work. Chromatographic separation of all derivatives was achieved by a Restek Rtx-5Sil MS (30 m, 0.25 mm I.D., 0.25 μm thickness) column using the following three temperature programs. For the analyses of phenols: initial temperature, 70 °C, with a 1 min hold, oven temperature ramps, 30 °C/min from 70 to 150 °C, 5 °C/min from 150 to 190 °C, 15 °C/min from 190 to 270 °C, and a 5 min hold at 270 °C. For the analyses of estrogens: initial temperature, 70 °C, with a 1 min hold, oven temperature ramps, 30 °C/min from 70 to 200 °C, 5 °C/min from 200 to 260 °C, and 30 °C/min from 260 to 300 °C, and a 10 min hold at 300 °C. For the analyses of the acidic pharmaceuticals: initial temperature, 70 °C, with a 1 min hold, oven temperature ramps, 30 °C/min from 70 to 190 °C, 5 °C/min from 190 to 260 °C, and 15 °C/min from 260 to 300 °C, with a 5 min hold at the final temperature.

The injection port temperature was 250 °C and the carrier gas (helium) flow rate was kept constant at 1 mL/min. Samples in 1 μL aliquots were injected in the pulsed splitless mode with a 1 min hold of injection pulse pressure at 30 psi. Electron-impact ionization mass spectrometry (EI/MS) was used for the detection of phenols and acids. Full-scan mass spectra of the derivatives were obtained over a range of *m*/*z* 50–600. Selected ion monitoring (SIM) was employed for the quantitative analyses of the target compounds, and the characteristic ions used are listed in Tables 1 and 2.

3. Results and discussion

3.1. Optimization of the extraction procedure

The Oasis MAX cartridge was made up of a mixed-mode polymer sorbent with both reversed-phase and anion-exchange functionalities. When a water sample was acidified to pH 3 or less, phenols and acids were in the unionized forms and so they would be adsorbed by the reversed-phase interactions similar to an ODS cartridge. In order to take advantage of its anion-exchange properties, the MAX cartridge was washed, at the end of extraction, by a base to convert the acids into their anionic forms so that they would be strongly attached to the NR_3^+ groups in the sorbent. While in some procedures a strong base such as NaOH was used, we have found that only a small quantity of a methanol/sodium acetate solution was sufficient for this conversion. Under such circumstances, selective elution of the phenols and acids in two fractions could be achieved. Elution of the cartridge with methanol removed only the less acidic phenols (along-

side other neutral coextractives) which were mainly adsorbed by the reversed-phase groups of the sorbent. For the elution of the more acidic pharmaceuticals, a mixture of 2% formic acid in methanol was required to convert the anions back to unionized forms. Perhaps due to the fact that TCS, being a trichlorophenol, is rather strongly acidic, we were unable to completely elute it from the MAX cartridge with methanol, even if the volume of the eluant was increased from 5 to 10 mL. The splitting of TCS in the two elution fractions varied with the sample matrix. Up to 30 and 65% of the total TCS could be found in the acidic drug fraction in influent samples and effluent (or spiked distilled water) samples, respectively. It was therefore necessary to quantitate this ubiquitous anti-bacterial agent in the phenol as well as the acidic pharmaceutical fractions.

In the early stages of the methods development, very low (<30%) recovery of the more volatile phenols, particularly the parabens, were observed. Since the recovery of the less volatile phenols such as TCS and BPA in the same samples was not affected, losses in the evaporation steps were initially suspected and later confirmed. Indeed, the addition of a small amount of a base such as methanolic NaOAc to the phenol fraction prior to the evaporation steps was enough to avert such losses. Similarly, evaporative losses (up to 80%) of clofibric acid, ibuprofen, and salicylic acid but not the other acidic pharmaceuticals were observed. This was unexpected as we had never observed such losses in our earlier procedure using the Oasis HLB cartridge [13]. The losses were apparently due to the presence of formic acid in the pharmaceutical fraction in the present procedure and were again effectively reduced by the addition of a base (NH_4OH in methanol) prior to the evaporation steps.

3.2. Derivatives of phenols and acidic pharmaceuticals

For better chromatography as well as improved selectivity and sensitivity in detection, phenols are often derivatized prior to GC/MS analysis. While numerous forms of derivatives have been reported, the pentafluoropropionyl derivatives were used in this work due to their rapid and quantitative reactions, stable products, good EI/MS sensitivity, and excellent chromatographic properties for a wide variety of phenols. In our previous work, it was shown that PFP derivatives of the alkylphenols [20], BPA [11], TCS [20], and estrogens [12] were easily formed by a 20 min, room temperature reaction between PFPA and the phenols. In this work, we have further extended the application to the determination of other phenols such as 2-phenylphenol and the four parabens.

At the completion of SPE, a small amount of water often remained on the cartridge even though the cartridge had been dried under vacuum for a few minutes. When the cartridge was eluted with methanol, water was also eluted alongside the phenols. As the presence of water and methanol in the extract would adversely affect the formation of PFP derivatives of all phenols, they had to be removed before derivatization took place. Their complete removal was achieved by repeated evaporation with the addition of methyl *tert*-butyl ether to the extract as described in the procedure.

Under the GC conditions used, the retention times of the PFP derivatives of *n*-propylparaben (7.06 min) and 2-phenylphenols (7.09 min) were very similar and they coeluted as a result. However, the coelution did not cause any cross interference in quantitation as each had its own unique characteristic ions (Table 1). The PFP derivatives of E_2 and E_2-d_3 also coeluted; again, their unique characteristic ions permitted the quantitation of each compound without interference.

Each of the acidic pharmaceuticals reacted with MTBSTFA at 75 °C to form a single product, with a mass spectrum indicating the formation of the *tert*-butyldimethylsilyl (TBDMS) derivative. While the molecular ions of these TBDMS derivatives are weak in many cases, they invariably have dominant $[M-57]^+$ or $[M-C_4H_9]^+$ ions which are characteristic of the parent compounds (Table 2). Indeed, in this work the $[M-57]^+$ ions were the base peaks in the EI mass spectra of the TBDMS derivatives for all but three acidic pharmaceuticals, namely clofibric acid, gemfibrozil, and indomethacin. For this reason, the TBDMS derivatives are a better choice than the TMS derivatives for the quantitative measurement of the acidic pharmaceuticals using selected ion monitoring. As salicylic acid has two active hydrogens on its molecule, reactions at lower temperatures or for shorter times would result in two products. Under such conditions, an intermediate product with only one TBDMS group was also observed due to incomplete silyation of the acid.

3.3. Method performance: accuracy, precision, and method detection limits

After the evaporative losses of the volatile phenols and acids were identified and the problem was resolved, the mean recovery of all compounds from fortified distilled water samples was found to be acceptable (between 87 and 110%) at two spiking levels ranging from 10 to 0.01 μg/L (Table 3). Standard deviations for these replicate determinations ($n = 4$) were lower than 7%, which indicated acceptable precision of the present procedure. Recovery of the surrogates from sewage samples was also within the range of 75–115%. Based on a concentration factor of 1000, the estimated method detection limit (MDL) was 0.1 μg/L for NP and 0.01 μg/L for the other phenols [10,11] and acidic pharmaceuticals [13]. The MDL for E_1 and E_2 [12] was 0.001 μg/L, with a concentration factor of 4000.

3.4. Phenolic compounds in municipal wastewater samples

Influent and effluent samples, collected from eight municipal sewage treatment plants located in southern Ontario, Canada, were tested for phenols and acidic pharmaceuticals. Contaminants originating from the use of household and industrial detergents, i.e. NP, OP, BPA, and TCS, were detected in all influent (Fig. 1) and effluent (Fig. 2) samples.

Table 3
Percent recovery of phenols and acidic pharmaceuticals from spiked distilled water samples

Compound	Spiking level (μg/L)	Mean ± SD (%)	Spiking level (μg/L)	Mean ± SD (%)
Methyl paraben	1.0	87 ± 4	0.1	89 ± 6
Ethyl paraben	1.0	92 ± 4	0.1	93 ± 5
Propyl paraben	1.0	96 ± 4	0.1	96 ± 4
Butyl paraben	1.0	99 ± 5	0.1	93 ± 5
2-Phenylphenol	2.5	96 ± 2	0.25	93 ± 4
4-*tert*-Octylphenol	1.0	94 ± 3	0.10	95 ± 5
4-Nonylphenol	10.0	102 ± 5	1.0	104 ± 6
4-*n*-Nonylphenol	0.5	95 ± 3	0.5	93 ± 4
Bisphenol A	1.0	98 ± 3	0.1	99 ± 5
Bisphenol A-d_{16}	0.5	99 ± 3	0.5	102 ± 4
Estrone (E_1)	0.10	105 ± 6	0.010	109 ± 7
17ß-Estradiol (E_2)	0.10	89 ± 5	0.010	95 ± 5
E_2-d_3	0.050	90 ± 4	0.050	91 ± 5
Clofibric acid	1.0	98 ± 3	0.1	94 ± 5
Ibuproffen	1.0	87 ± 4	0.1	86 ± 4
Salicylic acid	1.0	97 ± 3	0.1	95 ± 6
Gemfibrozil	1.0	99 ± 4	0.1	97 ± 5
Fenoprofen	1.0	98 ± 3	0.1	96 ± 4
Naproxen	1.0	101 ± 3	0.1	103 ± 5
Triclosan	1.0	93 ± 5	0.1	96 ± 5
Ketoprofen	1.0	102 ± 4	0.1	105 ± 6
Tolfenamic acid	1.0	99 ± 5	0.1	96 ± 4
Diclofenac-sodium	1.0	99 ± 3	0.1	104 ± 6
Indomethacin	1.0	107 ± 5	0.1	110 ± 7
2,3-D	2.5	93 ± 3	2.5	98 ± 5

Means of four replicates and standard deviations.

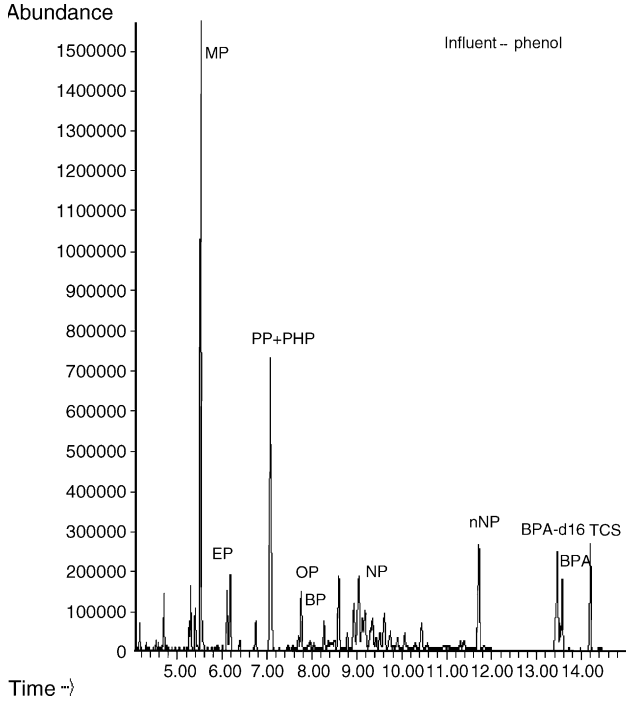

Fig. 1. A GC/MS/SIM chromatogram of a sewage influent extract, showing the phenols as their PFP derivatives.

Among the phenols included in this study, NP was found at the highest concentrations in the sewage samples (Table 4). It ranged from 2.72 to 25.0 μg/L (median 14.6 μg/L) in the influent and from 0.32 to 3.21 μg/L (median 0.70 μg/L) in the effluent. OP, BPA, and TCS were present at lower levels, with median concentrations of 3.08, 1.28, and 1.35 μg/L, respectively, in influent samples, and 0.06, 0.18, and 0.14 μg/L, respectively, in effluent samples. A comparison of these median concentrations suggested a >90% reduction of the alkylphenols and TCS in the final effluent. As indicated

Table 4
Concentrations of selected phenols in sewage influent and effluent samples

Sewage treatment plant[a]	MP (μg/L)	EP (μg/L)	PP (μg/L)	BP (μg/L)	PHP (μg/L)	OP (μg/L)	NP (μg/L)	BPA (μg/L)	TCS (μg/L)	E_1 (μg/L)	E_2 (μg/L)
A influent	0.63	0.12	0.86	0.12	5.19	3.24	18.6	1.45	0.87	0.052	0.003
A effluent	0.02	<0.01	<0.01	<0.01	0.10	0.04	0.64	0.13	0.11	0.011	0.002
B influent	0.24	0.08	1.25	0.11	0.72	2.84	24.9	1.10	1.81	0.015	0.013
B effluent	0.04	<0.01	0.02	<0.01	0.03	0.02	0.54	0.07	0.13	0.027	0.002
C influent	0.10	0.02	0.24	0.02	1.57	3.56	11.4	2.40	1.43	0.010	0.010
C effluent	0.03	<0.01	0.01	<0.01	<0.01	0.04	0.88	0.23	0.24	0.002	<0.001
D influent	0.21	0.04	0.60	0.11	0.27	3.35	25.0	0.69	1.83	0.008	0.014
D effluent	0.05	<0.01	<0.01	<0.01	<0.01	0.01	0.32	0.02	0.08	<0.001	<0.001
E influent	0.69	0.14	0.48	0.12	4.48	2.98	17.9	2.15	1.55	0.018	0.005
E effluent	<0.01	<0.01	0.01	0.01	0.05	0.32	3.21	0.45	0.17	0.005	0.002
F influent	0.60	0.07	0.40	0.07	3.58	3.18	9.70	0.58	1.26	0.011	0.007
F effluent	0.04	<0.01	0.01	<0.01	<0.01	0.17	0.70	0.25	0.19	0.002	<0.001
G influent	0.91	0.16	1.40	0.13	5.32	1.59	6.24	2.02	1.11	0.039	0.022
G effluent	0.03	<0.01	0.01	0.01	0.14	0.47	2.34	0.31	0.36	0.054	0.002
H influent	1.47	0.27	2.43	0.26	0.76	0.38	2.72	0.21	0.90	0.016	0.005
H effluent	0.04	<0.01	0.04	<0.01	0.03	0.09	0.70	0.04	0.05	0.005	<0.001
Minimum (influent)	0.10	0.02	0.20	0.02	0.27	0.38	2.72	0.21	0.87	0.008	0.003
Maximum (influent)	1.47	0.27	2.43	0.26	5.32	3.56	25.0	2.40	1.83	0.052	0.022
Median (influent)	0.62	0.10	0.73	0.11	2.58	3.08	14.6	1.28	1.35	0.016	0.009
Minimum (effluent)	0.02	<0.01	<0.01	<0.01	<0.01	0.01	0.32	0.02	0.05	<0.001	<0.001
Maximum (effluent)	0.03	<0.01	0.04	0.01	0.14	0.47	3.21	0.45	0.36	0.054	0.002
Median (effluent)	0.05	<0.01	0.01	<0.01	0.05	0.06	0.70	0.18	0.14	0.005	0.002

[a] Located in southern Ontario, Canada (see Section 2).

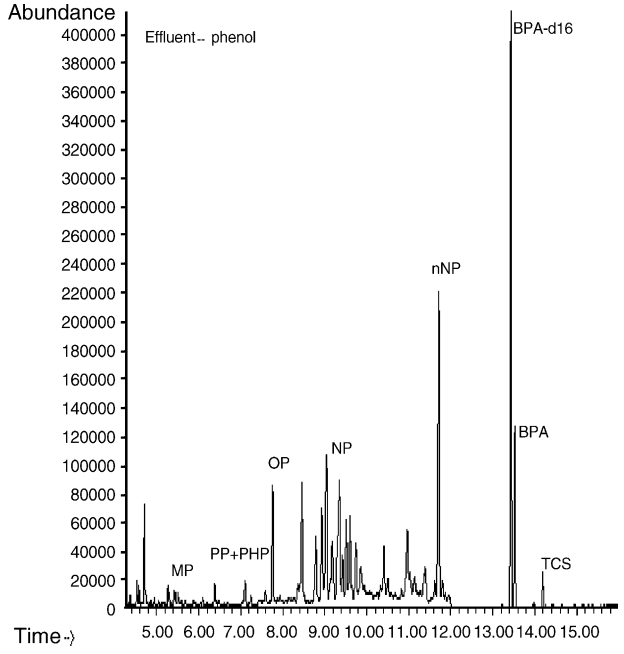

Fig. 2. A GC/MS/SIM chromatogram of a sewage effluent extract, showing the phenols as their PFP derivatives.

by the elevated levels of these contaminants in the sludge [21], this huge reduction appeared to be mainly the results of accumulation onto sludge rather than biodegradation of these lipophilic compounds.

In contrast to the alkylphenols, BPA, and TCS, there were very few reports on the occurrence of the parabens and 2-phenylphenol in the Canadian environment. Parabens have long been used as preservatives in foodstuffs, cosmetics, and personal-care products. However, these compounds are also referred to as endocrine-disrupting chemicals, as they all exhibit estrogenic activities. Recently, parabens have been found, at ng/g levels by LC/MS/MS, in human breast tumors [22]. It has then led to the speculation that regular application of paraben-containing personal-care products to the underarm and breast area could influence breast cancer development. Methyl, ethyl, *n*-propyl, and *n*-butyl parabens were detected in all influent samples, with the *n*-propyl paraben being the most abundant chemical in the samples (with concentrations from 0.2 to 2.43 μg/L, median 0.73 μg/L). In contrast, these parabens were virtually absent in the effluent samples as their levels were either close to or below their detection limit of 0.01 μg/L (Table 4).

2-Phenylphenol, commonly used as a surface disinfectant in hospitals, nursing homes, commercial laundries, and food processing plants, was also detected in the same samples.

Again, the observed concentrations of 2-phenylphenol were much higher in the influent (median 2.58 μg/L) than the effluent (median 0.05 μg/L).

The occurrence of E_2, the most potent female hormone, and of its major metabolites has been reported in sewage samples [12,14]. As these compounds were extracted, eluted, and derivatized alongside the other phenols, the same general procedure could have been used for their determination. However, in order to achieve a detection limit of 0.001 μg/L for the estrogens, a final volume of 250 μL (equivalent to a concentration factor of 4000 based on a 1 L sample) for a sample extract was needed in the procedure. Similar to the previous findings, the observed median concentrations for E_2 and E_1 in the influent samples were 0.009 and 0.016 μg/L, respectively. Due to the rapid degradation of E_2 and E_1 under aerobic conditions, their median concentrations in the effluent samples were further reduced to 0.002 and 0.005 μg/L, respectively.

3.5. Acidic pharmaceuticals in municipal wastewater samples

Seven acidic pharmaceuticals, namely, ibuprofen, salicylic acid, gemfibrozil, naproxen, ketoprofen, diclofenac, and indomethacin, were consistently detected in the influent and effluent samples (Table 5). In contrast, clofibric acid, fenoprofen and tolfenamic acid have not been detected in this set of samples. As mentioned earlier, the anti-bacterial agent triclosan is split between the phenol and acid fractions and it is therefore necessary to quantitate this compound in both fractions.

Ibuprofen, salicylic acid, and naproxen were the more abundant acidic pharmaceuticals in the influent (Fig. 3), with respective median concentrations of 6.77, 6.86, and 2.76 μg/L in the samples. For the effluent (Fig. 4), naproxen and ibuprofen were detected at higher levels than the other drugs, with respective median concentrations of 0.82 and

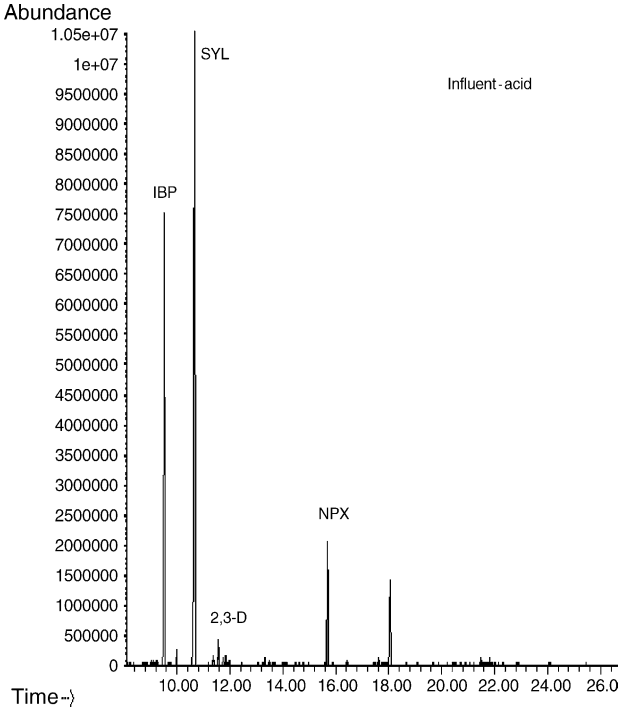

Fig. 3. A GC/MS/SIM chromatogram of a sewage influent extract, showing the acidic pharmaceuticals as their TBDMS derivatives.

Table 5
Concentrations of selected acidic pharmaceuticals in sewage influent and effluent samples

Sewage treatment plant[a]	IBP (μg/L)	SYL (μg/L)	GFB (μg/L)	NPX (μg/L)	KTP (μg/L)	DCF (μg/L)	IDM (μg/L)
A influent	7.16	8.03	0.30	1.73	0.06	0.05	0.43
A effluent	0.22	0.12	0.08	0.46	0.06	0.10	0.49
B influent	10.21	5.76	0.14	6.03	0.08	0.18	0.42
B effluent	0.17	0.09	0.15	0.36	0.04	0.08	0.36
C influent	4.10	10.2	0.38	2.84	0.11	0.15	0.22
C effluent	0.12	0.32	0.24	0.95	0.06	0.12	0.14
D influent	7.67	7.96	0.20	2.43	0.06	0.09	0.13
D effluent	0.11	0.01	0.19	0.69	0.04	0.08	0.08
E influent	4.63	2.82	0.27	1.88	0.09	0.19	0.16
E effluent	2.17	0.23	0.29	2.31	0.06	0.16	0.17
F influent	6.37	4.91	36.53	2.94	0.15	2.45	0.33
F effluent	0.62	0.15	2.09	2.54	0.09	0.25	0.18
G influent	4.95	3.78	0.24	3.45	0.07	0.18	0.36
G effluent	0.50	0.11	0.19	1.57	0.04	0.16	0.18
H influent	7.48	12.7	0.12	2.68	0.07	0.08	0.03
H effluent	0.39	0.16	0.09	0.69	0.04	0.07	0.04
Minimum (influent)	4.10	2.82	0.12	1.73	0.06	0.05	0.03
Maximum (influent)	10.21	12.7	36.53	6.03	0.15	2.45	0.43
Median (influent)	6.77	6.86	0.26	2.76	0.08	0.17	0.28
Minimum (effluent)	0.11	0.01	0.08	0.36	0.04	0.07	0.04
Maximum (effluent)	2.17	0.32	2.09	2.54	0.09	0.25	0.49
Median (effluent)	0.31	0.14	0.19	0.82	0.05	0.11	0.18

[a] Located in southern Ontario, Canada (see Section 2).

0.31 μg/L. These concentrations suggest a >85% removal of the two drugs by the sewage treatment processes. The relatively low concentrations of salicylic acid found in the effluent samples were probably due to the instability of this compound in sewage samples [13,23]. The levels of the acidic pharmaceuticals found in this study have further confirmed our previous findings on the same compounds in sewage [13], as those results were obtained with a different procedure using the Oasis HLB cartridge and the formation of the trimethylsilyl (TMS) derivatives of the acidic drugs.

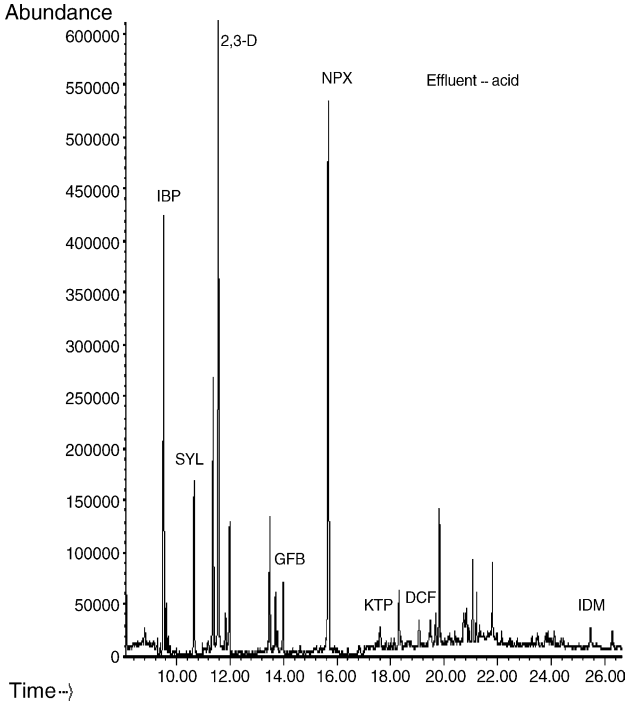

Fig. 4. A GC/MS/SIM chromatogram of a sewage effluent extract, showing the acidic pharmaceuticals as their TBDMS derivatives.

3.6. Conclusions

A multi-residue method has been developed and validated for the determination of selected acidic pharmaceuticals, personal-care products, and a wide variety of phenols with endocrine-disrupting potentials in sewage samples. For compounds such as phenols and acidic PPCPs, the use of a cartridge with anion-exchange groups for extraction has provided better selectivity than those with the more commonly used C_{18} groups. Elution of phenols and acids into two separate groups has produced an extract with less coextractives in each fraction, which would be beneficial for complex matrices such as sewage samples. This procedure has also permitted phenols and acids to be converted into different derivatives, so that further enhancement in selectivity and sensitivity could be made in the final analysis. When this method was applied to influent and effluent samples, results for alkylphenols, BPA, estrogens, and acidic drugs obtained in this study were consistent with those reported in previous works using individual methods. This multi-residue procedure is therefore a useful and more efficient alternative to the previous methods, especially when results for multiple parameters are required. The application of this method has generated some of the first results of methyl, ethyl, propyl, and butyl parabens as well as 2-phenylphenol in Canadian sewage samples.

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