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Determination of Alkylphenols, Bisphenol A, Steroids and Sterols in Surface and Waste Water Samples using Gas Chromatography with Mass Spectrometry (GC/MS and GC/MS/MS)

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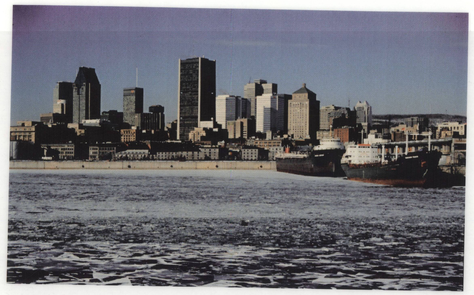


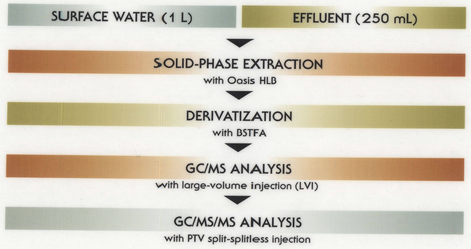
ABSTRACT

Endocrine-disrupting compounds have become a cause for concern for aquatic systems in the last decade. The identification and quantification of these contaminants at trace levels in environmental samples is of prime importance to linking biota exposure with observed estrogenic and androgenic effects. To date, Canadian water quality guidelines have only been established for nonylphenol and its ethoxylates (NPEs), at a rate of 1 µg/L teq (toxic equivalent). On June 23, 2001, the federal departments of Environment and Health recommended that NPEs be added to the List of Toxic Substances under the *Canadian Environmental Protection Act 1999* (CEPA 1999). The final decision to declare NPEs as toxic followed the publication of a draft assessment report on the Priority Substances List, which concluded that these substances were harmful to the environment. A method was developed for the determination and quantification of alkylphenols (4-nonylphenol and 4-*tert*-octylphenol), bisphenol A, steroids (estradiol-17β, estriol, estrone, hydrocortisone, testosterone, 17α-ethynyl-estradiol) and sterols (cholesterol, coprostan-3-ol, coprostan and coprostan-3-one) in surface and waste water by GC/MS and GC/MS/MS. Percentage recoveries were above 60% for the majority of selected compounds, with quantification limits being between 0.5 to 40 ng/L. Concentrations ranging from ng/L to µg/L were detected. Coprostan was detected at a concentration of 20 ng/L in surface water; bisphenol A was found at 450 µg/L in waste water.

OBJECTIVES

The objectives of this work were to develop GC/MS and GC/MS/MS methods with BSTFA as the derivatization agent with large-volume and PTV split-splitless injection, respectively, and to detect target compounds in surface and waste waters.

ANALYTICAL METHOD



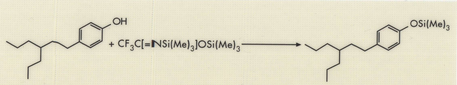


Figure 1 An example of a derivatization with BSTFA for 4-nonylphenol

RESULTS AND DISCUSSION

- Since all alkylphenols, bisphenol A, steroids and sterols selected for this study contain hydroxyl groups (except for coprostan), a derivatization step (in this case, a silylation reaction) was required prior to GC analysis. BSTFA was selected because of its rapid reactivity with compounds containing hydroxyl groups, its high volatility resulting in the non-coelution of early eluting peaks, and the high volatility, stability and good solubility of the derivatized compounds.
- The calibration curve was linear for all compounds in GC/MS and GC/MS/MS ($R^2 > 0.995$).
- Quantification limits (QL) are sufficient (0.5 and 40 ng/L) for detecting the target compounds in surface and waste waters.
- Relative standard deviation (RSD) was less than 10%, showing the good repeatability of LVI.
- Percentage recoveries for this method were above 60% for the majority of selected compounds, except for alkylphenols, for which percentage recoveries were some 25% for 4-*tert*-octylphenol and roughly 50% for 4-nonylphenol. Both of these products are phenolic compounds, however, and they may be better extracted at levels pH < 5. The pH of both surface and waste waters was approximately 7.

Table 1 Retention times and m/z for GC/MS analysis

Compounds				
No.	Name	Retention time	Molecular mass	*m/z* used for quantification
1	4-*tert*-octylphenol	19.14	206.3	207
2	4-nonylphenol	28.14	220.4	179, 292
3	Bisphenol A	38.68	228.3	357
4	Bisphenol A-d_{16}	38.94	244.3	368
5	Estrone	48.08	270.4	218, 257, 342
6	Estradiol-17β	48.94	288.4	285, 326, 416
7	Testosterone	49.13	288.4	226, 270, 360
8	17-α-ethynylestradiol	50.17	296.4	285, 425
9	Coprostan	51.97	372.7	217, 357
10	Estriol	53.58	272.4	311, 386, 414
11	Coprostan-3-ol	57.13	388.7	215, 355, 370
12	Coprostan-3-one	58.76	386.7	161, 316
13	Cholesterol	59.07	386.7	329, 353, 369

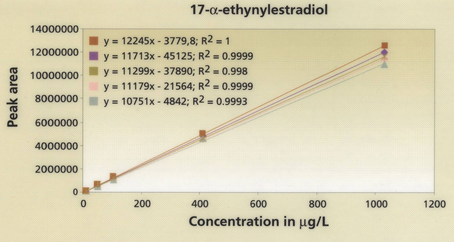
Table 2 Retention times and selected ions used for GC/MS/MS analysis

Segment Time (min)	Retention time	Compounds	Selected ions	Daughter ions	MS/MS full scan interval
8.0–18.0			50-650		
18.1–26.0	18.55	4-*tert*-octylphenol	207	151, 163, 179	50-206
26.1–35.5	27.66	4-nonylphenol	179	73	50-180
35.6–47.0	36.27	Bisphenol A	357	191, 267, 357	197, 368
	36.50	Bisphenol A-d_{16}	368	197, 368	50-370
47.1–51.0	48.12	Estrone	342	242, 257	50-350
	48.61	Estradiol-17β	285	229, 256, 269	50-300
	49.00	Testosterone	226	198, 211	50-220
	50.82	17-α-ethynylestradiol	425	193, 231, 407	200-440
51.1–52.5	51.75	Coprostan	217	121, 147, 161	50-220
52.6–56.0	53.09	Estriol	414	295, 311, 324	50-420
56.1–58.0	56.23	Coprostan-3-ol	355	187, 219, 243	50-260
	58.24	Coprostan-3-one	161	119, 133, 145	50-165
	58.24	Cholesterol	329	203, 217, 313, 328	190-350

Table 3 Mean recovery values and relative standard deviations (RSD, n = 3, 3 levels) of alkylphenols, bisphenol A, steroids and sterols

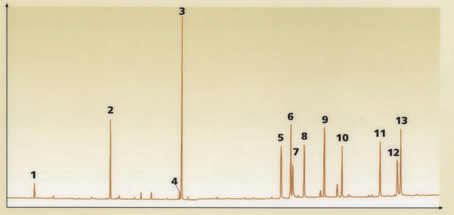
Compounds	Recoveries (%)	RSD (%)	QL for LVI/GC/MS (ng/L)	QL for GC/MS/MS (ng/L)
4-*tert*-octylphenol	25	4	5	1
4-nonylphenol	50	10	2	1
Bisphenol A	78	4	0.5	0.5
Estrone	96	3	10	2
Estradiol-17β	104	4	2	3
Testosterone	97	3	20	25
17-α-ethynylestradiol	98	2	5	20
Coprostan	44	18	10	5
Estriol	90	19	10	12
Coprostan-3-ol	95	22	10	30
Coprostan-3-one	94	16	15	5
Cholesterol	66	4	15	40

Note: • Determined from spiked distilled water using SPE with Oasis HLB cartridges followed by derivatization with BSTFA and GC/MS analysis;
• Spiking levels: 50, 200 and 1000 µg/L.



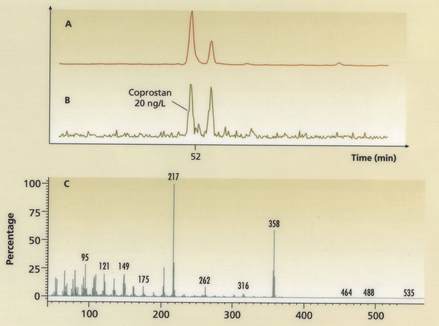
Note: Injected volumes: 40 µL.

Figure 2 Calibration curves from independently prepared replicates of five 17-α-ethynylestradiol standard solutions in derivatized form with BSTFA from LVI/GC/MS data



Note: • Injected volume: 40 µL.
• See Table 1 for peak assignments and *m/z* values.

Figure 3 LVI/GC/MS full-scan chromatogram of a mixture of alkylphenols, bisphenol A, steroids and sterols with approximately 400 ng/µL of each compound in derivatized form with BSTFA



Note: (A) GC/MS chromatogram in TIC mode;
(B) Reconstructed ion chromatogram (*m/z*: 217 + 358);
(C) Identification of coprostan by its mass spectrum.

Figure 4 Identification of coprostan in a derivatized river sample extract using LVI/GC/MS (40 µL)

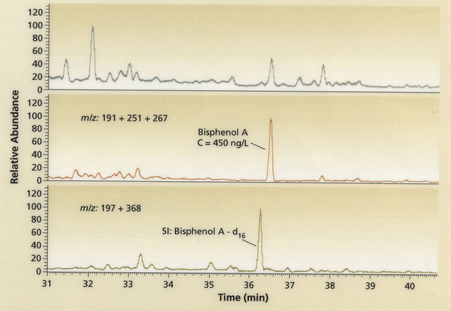


Figure 5 Confirmation by GC/MS/MS of bisphenol A in a derivatized extract from a waste water sample

CONCLUSION

It is possible, using the methods described here, to determine various contaminants in environmental samples at concentration levels at which endocrine effects may be induced. Coprostan was detected at a concentration of 20 ng/L in surface water, and bisphenol A was found at a concentration of 450 ng/L in waste water.

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