Application Note: 466

Detection of Pharmaceuticals, Personal Care Products, and Pesticides in Water Resources by APCI-LC-MS/MS

Liza Viglino[1], Khadija Aboulfald[1], Michèle Prévost[2], and Sébastien Sauvé[1]
[1]Department of Chemistry, Université de Montréal, Montréal, QC, Canada; [2]Département of Civil, Geological, and Mining Engineering, École Polytechnique de Montréal, Montréal, QC, Canada

Key Words

- TSQ Quantum Ultra
- Water Analysis
- Solid Phase Extraction

Introduction

Pharmaceuticals (PhACs), personal care product compounds (PCPs), and endocrine disruptors (EDCs), such as pesticides, detected in surface and drinking waters are an issue of increasing international attention due to potential environmental impacts[1,2]. These compounds are distributed widely in surface waters from human and animal urine, as well as improper disposal, posing a potential health concern to humans via the consumption of drinking water. This presents a major challenge to water treatment facilities.

Collectively referred to as organic wastewater contaminants (OWCs), the distribution of these emerging contaminants near sewage treatment plants (STP) is currently an area of investigation in Canada and elsewhere[3,4]. More specifically, some of these compounds have been detected in most effluent-receiving rivers of Ontario and Québec[5,6]. However, it is not clear whether contamination is localized to areas a few meters from STP discharges or whether these compounds are distributed widely in surface waters, potentially contaminating sources of drinking water.

A research project at the University of Montreal's Chemistry Department and Civil, Geological, and Mining Engineering Department was undertaken to establish the occurrence and identify the major sources of these compounds in drinking water intakes in surface waters in the Montreal region. The identification and quantification of PhACs, PCPs, and EDCs is critical to determine the need for advanced processes such as ozonation and adsorption in treatment upgrades.

The establishment of occurrence data is challenging because of: (1) the large number and chemical diversity of the compounds of interest; (2) the need to quantify low levels in an organic matrix; and (3) the complexity of sample concentration techniques. To address these issues, scientists traditionally use a solid phase extraction (SPE) method to concentrate the analytes and remove matrix components.

After extraction, several different analytical techniques may perform the actual detection such as GC-MS/MS and more recently, LC-MS/MS[7,8]. Another analytical challenge resides in the different physicochemical characteristics and wide polarity range of organic compounds – making simultaneous preconcentration, chromatography separation, and determination difficult. Analytical methods capable of detecting multiple classes of emerging contaminants would be very useful to any environmental monitoring program. However, up to now, it has often been a necessity to employ a combination of multiple analytical techniques in order to cover a wide range of trace contaminants[9]. This can add significant costs to analyses, including equipment, labor, and time investments.

Goals

To develop a simple method for the simultaneous determination of trace levels of compounds from a diverse group of pharmaceuticals, pesticides, and personal care products using SPE and liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Determine which selected substances are present in significant quantities in the water resources around the Montreal region.

Materials and Method

Analyte selection

Compounds were selected from a list of the most-frequently encountered OWCs in Canada[4-6] (Figure 1).

Sample collection

Raw water samples were taken from the Mille Iles, des Prairies, and St-Laurent rivers. Three samples were collected at the same time from each river in pre-cleaned, four-liter glass bottles and kept on ice while being transported to the laboratory. These water sources vary widely due to wastewater contamination and sewer overflow discharges.

All samples were acidified with H_2SO_4 for sample preservation and stored in the dark at 4 °C. Immediately before analysis, samples were filtered using 0.7 µm pore-size fiberglass filters followed by 0.45 µm pore size mixed-cellulose membranes (Millipore, MA, USA). Samples were extracted within 24 hours of collection.

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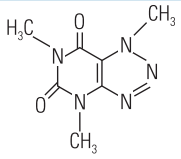
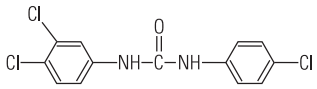
Personal Care Products

Caffeine (CAF)
MW: 194.19, pKa =10.4,
Stimulant

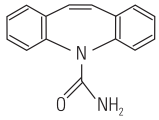


Triclocarban (TCC)
MW: 315.19,
Anti-bacterial agent

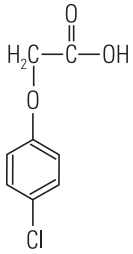


Pharmaceuticals

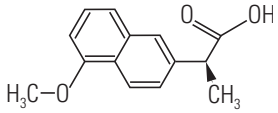
Carbamazepine (CBZ)
MW: 236.27, pKa=13.9,
Anticonvulsant



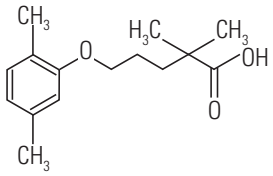
Clofibric acid
MW: 214.65
Metabolite lipid regulator



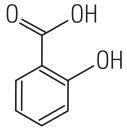
Naproxen (NAPRO)
MW: 230.26, pKa=4.15
Analgesic



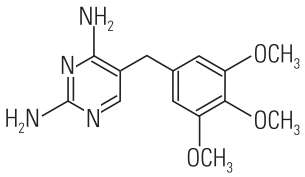
Gemfibrozil (GEM)
MW: 250.33,
Anti-cholesterol



Salicylic acid
MW: 138.12
Metabolite of acetylsalicylic acid (aspirin)

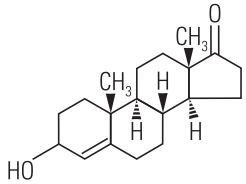


Trimethoprim (TRI)
MW: 290.30, pKa=7.12
Anti-infective

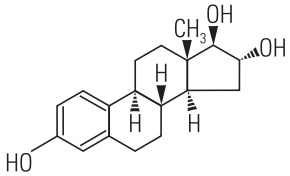


Hormones

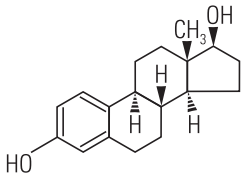
Estrone
MW: 270.4
Estrogen



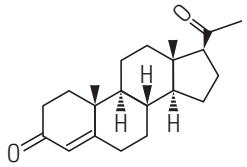
Estriol
MW: 288.4
Estrogen



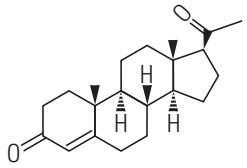
Estradiol
MW: 272.4
Estrogen



Progesterone
MW: 314.15
Progestogen



17-α-Ethinylestradiol
MW: 296.4
Synthetic estrogen



Pesticide

Atrazine (ATRA)
MW: 215.68,
pKa =1.7,
Herbicide

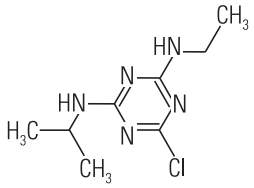


Figure 1: Molecular structures of selected compounds

Concentration and Extraction Procedure

The solid phase extraction procedure is illustrated in Figure 2. Briefly, analytes were concentrated and extracted using a 200 mg C18-like analytical cartridge. Retained analytes were eluted from the cartridges using 3 mL MTBE:MeOH 90/10 and 3 mL MeOH. They were then collected on the conical-bottom centrifuge tube for evaporation to dryness with N_2 (g). Extracted analytes were reconstituted to 200 µL with 90% water/formic acid 0.1% and 5% MeOH solution containing the internal standards.

LC-MS/MS conditions

HPLC separation was done with a Thermo Scientific Surveyor HPLC system. Separation conditions are given in Table 1. Detection and quantification of the analytes were performed with a Thermo Scientific TSQ Quantum Ultra triple stage quadrupole mass spectrometer using selective reaction monitoring (SRM) (Table 2). Preliminary experiments were performed with two atmospheric pressure ionization (API) sources – ESI and APCI – to detect all compounds. Although some compounds showed a slightly higher intensity with the ESI source (i.e. atrazine), APCI was selected because of the higher sensitivity provided for steroids. This endocrine disruption class is an important analytical challenge due to the low detection limits (1 ng/L) required for the determination of these compounds. These compounds are known to affect the living organisms at very low concentrations. Given that the aim was to develop a simple analytical method to detect as wide a range of compounds as possible, we selected the APCI source. The small loss in sensitivity for some easily measured molecules was more than compensated by the gain in sensitivity for other compounds that could not have been detected using ESI. Moreover, APCI ionization is known in some cases to be less susceptible to matrix interferences than ESI ionization[10]. Lastly, some authors demonstrated signal suppression for analysis of various organic waste compounds in water samples using ESI-LC-MS/MS[11].

The identification of analytes was confirmed by the LC retention time[12,13]. Instrument control and data acquisition were performed with Thermo Scientific Xcalibur software.

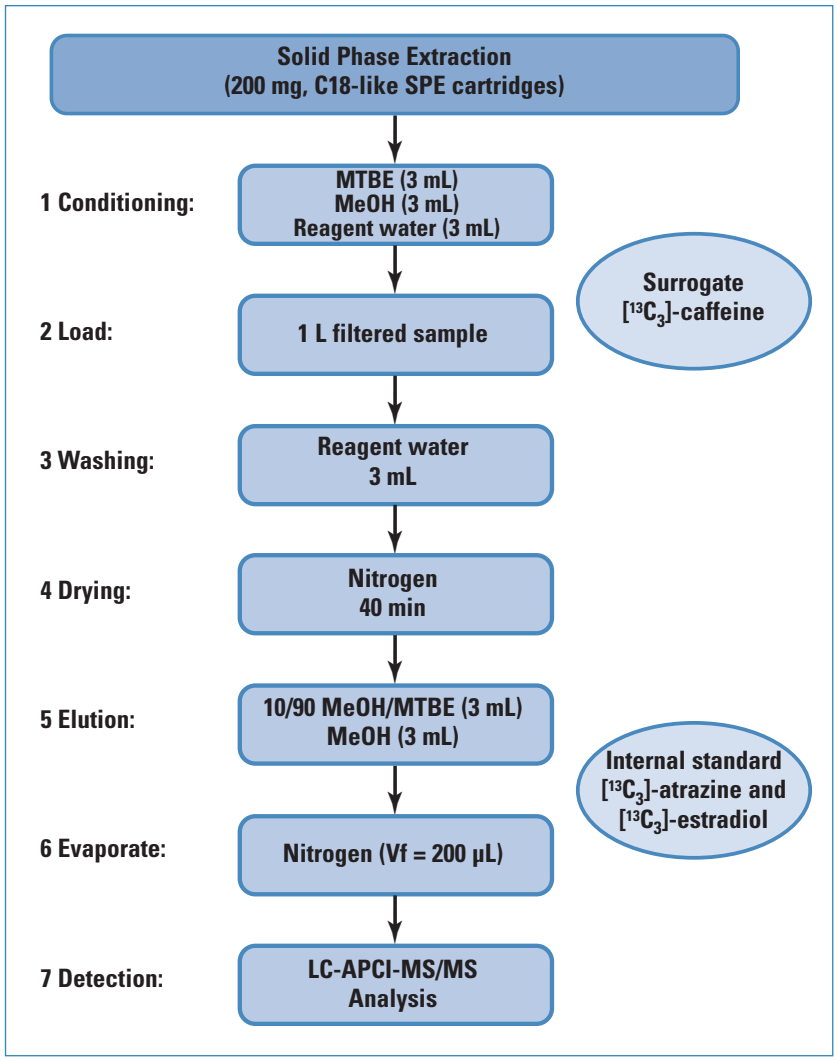


Figure 2: SPE enrichment procedure

Table 1: Instrument Parameters

HPLC		MS		
Column:	Thermo Scientific Hypersil GOLD (50 x 2.1 mm, 3 µm)	Ionization mode:	$APCI^+$	$APCI^-$
Column temperature:	30 °C	Discharge current:	3 µA	4 µA
Mobile phase A:	0.1% Formic acid/H_2O	Vaporizer temperature:	500 °C	500 °C
Mobile phase B:	MeOH	Capillary temperature:	250 °C	250 °C
Injection volume:	20 µL	Sheath gas pressure:	40 arb units	30 arb units
Flow rate:	500 µL/min	Aux. gas pressure:	20 arb units	15 arb units
Gradient:	T=0, A=90%, B=10%	Collision gas pressure:	1.5 mTorr	1.5 mTorr
	T=1, A=90%, B=10%	Source CID:	-10 V	15 V
	T=15, A=1%, B=99%			
	T=16.5, A=1%, B=99%			
	T=17, A=90%, B=10%			
	T=22, A=90%, B=10%			

Table 2: SRM transitions used for detection and quantification

Compound	Precursor ion (m/z)	Product ion (m/z)	CE (eV)	Tube lens (V)
Trimethoprim	291.16	230.16	22	90
Caffeine	195.10	138.10	18	77
Estriol	271.24	157.10	18	80
Carbamazepine	237.11	194.10	20	80
Atrazine	216.11	174.10	34	97
Naproxen	231.11	185.10	13	101
17-α-Ethinylestradiol	279.16	133.10	31	86
Estradiol	255.16	159.10	17	79
Estrone	271.24	157.10	18	80
Progesterone	315.26	109.10	38	118
TCC	316.99	127.04	32	99
Gemfibrozil	251.09	129.10	20	118
Salicylic acid*	137.04	93.10	31	72
Clofibric acid*	213.17	127.10	32	102

*APCI-

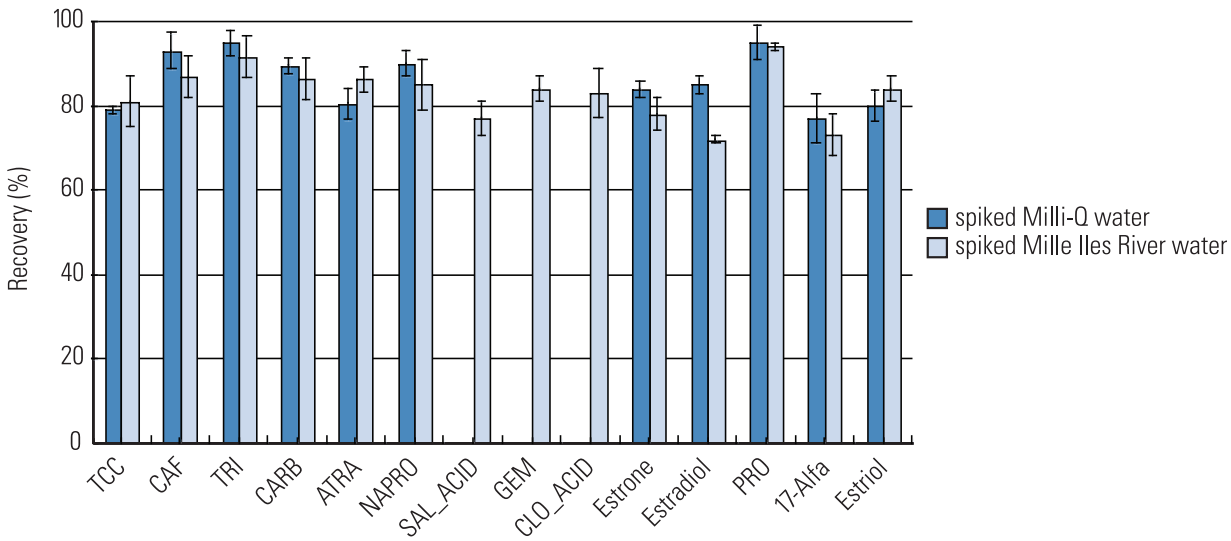


Figure 3: Mean recoveries for the extraction of selected compounds using C18-like cartridges (spiked in Milli-Q water and Mille Iles River water at 50 ng/L, n=6)

Results and Discussion

Reproducibility (%RSD), ranging from 3% to 11% for all analytes, was very good. Accuracy (recovery percentages), ranging between 72% to 94% for all compounds in spiked matrix, was satisfactory and indicated high performance of our method. Results are shown in Table 3.

Matrix effects are very important when developing an LC-MS/MS method and can affect reproducibility and accuracy[14]. This phenomenon was evaluated by comparing recovery percentages in Milli-Q® water and surface water samples (Mille Iles River) spiked at 50 ng/L (n = 6). We can consider a very low matrix effect in surface waters since signal suppression varies from 1% to 13%, except for atrazine and TCC showing an enhancement signal of 6% and 2%, respectively (Figure 3).

Good linearity in surface water samples was observed over a concentration range from <LOD to 100 ng/L with correlation coefficients greater than 0.99 for all compounds. Detection limits in surface water were in the range of 0.03 to 2 ng/L (Table 3).

The compounds of interest were investigated using samples from various surface waters. Figure 4 shows representative LC-MS/MS chromatograms of selected compounds in surface water. The concentrations are illustrated in Figure 5. The selected compounds were detected in all river samples at various concentrations depending on sampling locations (Figure 5 a and b). The highest concentrations were found for caffeine (16-24 ng/L), atrazine (1.5-39 ng/L), salycilic acid (10-33 ng/L) and gemfibrozil (4-14 ng/L). The lowest concentrations were found for carbamazepine (3-5 ng/L), clofibric acid, and two hormones (progesterone and estradiol). Trimethoprim, triclocarban and other selected hormones were detected at trace levels (Trace ≤ limit of detection).

Overall, concentrations of most of the compounds analysed were similar to those reported from other areas in Canada and Europe[3,4].

Table 3: Retention time, limit of detection (LOD), linearity, recoveries and RSD (%) data for each detected compounds in tap water.

Compound	Retention time (min)	LOD* (ng/L)	R^2**	Recovery***(%)	RSD (%)
Trimethoprim	5.46	0.50	0.9998	91	7
Caffeine	5.79	0.07	0.9995	87	9
Estriol	10.14	0.30	0.9981	84	9
Carbamazepine	10.76	0.09	0.9999	86	5
Atrazine	11.41	0.03	0.9995	86	3
Naproxen	12.62	2.00	0.9996	85	9
17-α-Ethinylestradiol	12.85	0.50	0.9931	73	10
Estradiol	12.88	0.10	0.9979	72	6
Estrone	12.94	0.60	0.9989	79	9
Progesterone	14.44	0.08	0.9994	94	4
TCC	15.10	0.20	0.9970	81	10
Gemfibrozil	15.17	2.00	0.9991	84	6
Salicylic acid	8.82	0.90	0.9993	77	6
Clofibric acid	12.00	0.60	0.9989	83	11

*LOD in surface water (Mille Iles River)
**Value for calibration line in river water (0-100 ng/L)
***Recoveries over the total method (surface samples spiked at 50 ng/L, n = 6).

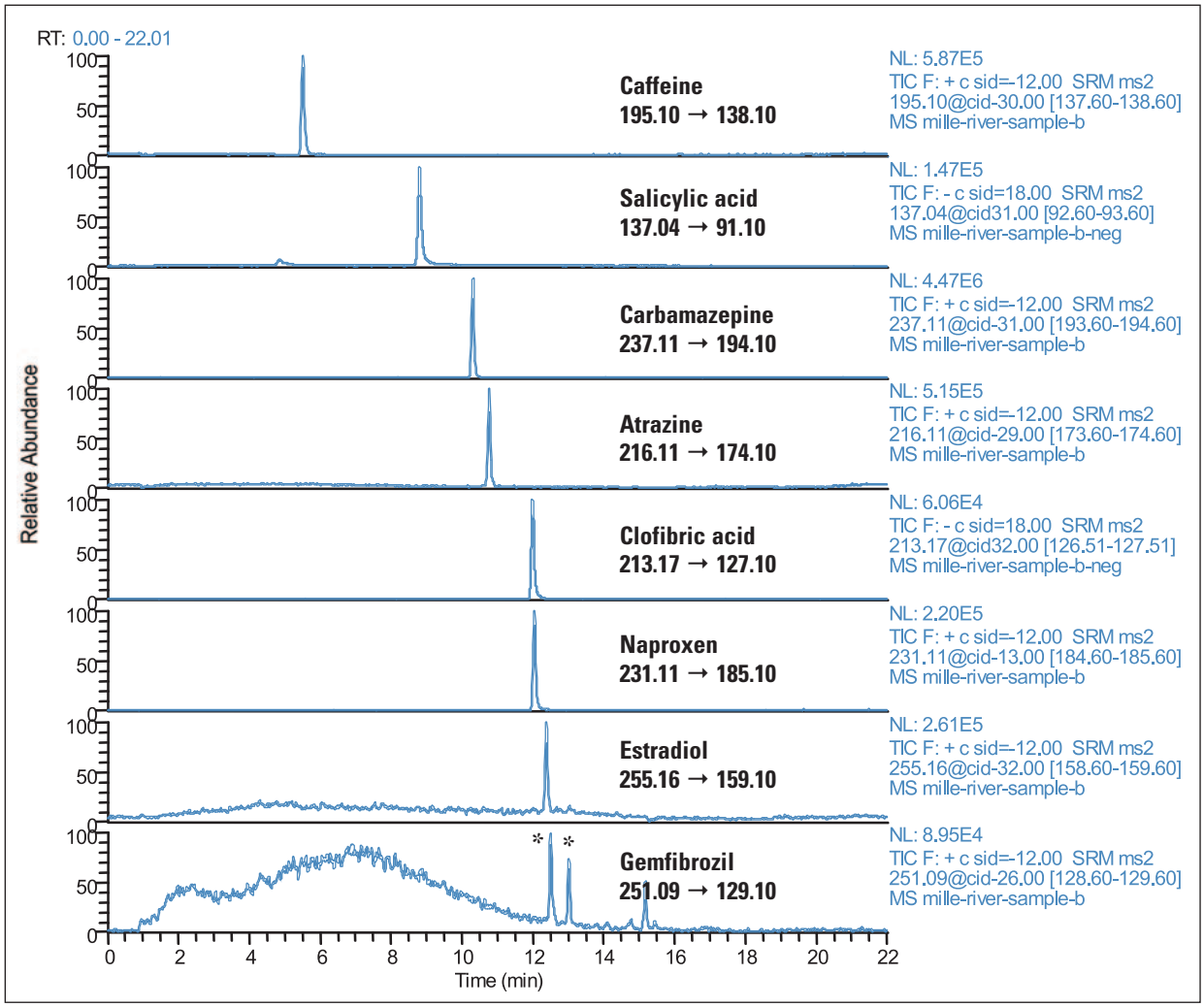


Figure 4: Representative SRM chromatograms of some selected compounds detected in water matrix (Mille Iles River). Peak due to interferences are marked by asterisks (*)

Conclusion

We developed and successfully applied an APCI-LC-MS/MS method for quantifying a wide range of compounds from a diverse group of pharmaceuticals, pesticides, and personal care products at concentration in the low ng/L range in surface waters with good precision and accuracy. Results confirmed the presence of pharmaceuticals, personal care products, and endocrine disruptors in all water resources around the region of Montreal. The concentrations of compounds fluctuated with sampling locations due to the variation of these sources, wastewater contamination and combined sewer overflow discharges.

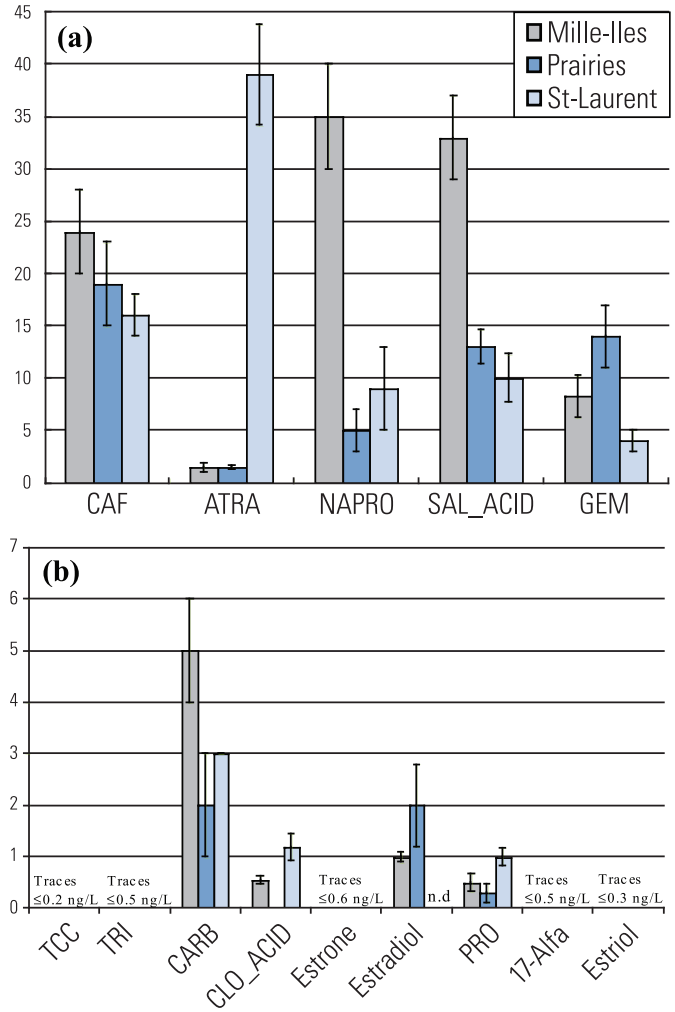


Figure 5: (a) The highest mean concentrations of selected compounds in water samples collected from Mille-Iles River, des Prairies River and St-Laurent River (n = 6). (b) The lowest mean concentrations of selected compounds in water samples collected from Mille Iles River, des Prairies River and St-Laurent River (n = 6).

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