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Automated on-line column-switching HPLC-MS/MS method with peak focusing for measuring parabens, triclosan, and other environmental phenols in human milk☆

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ABSTRACT

Parabens (esters of *p*-hydroxybenzoic acid) and triclosan are widely used as preservatives and antimicrobial agents, respectively, in personal care products, pharmaceuticals, and food processing. Because of their widespread use and potential risk to human health, assessing human exposure to these compounds in breastfed infants is of interest. We developed a sensitive method, using a unique on-line solid-phase extraction-high performance liquid chromatography-tandem mass spectrometry system with peak focusing feature, to measure in human milk the concentrations of five parabens (methyl-, ethyl-, propyl-, butyl-, and benzyl parabens), triclosan, and six other environmental phenols: bisphenol A (BPA); *ortho*-phenylphenol (OPP); 2,4-dichlorophenol; 2,5-dichlorophenol; 2,4,5-trichlorophenol; and 2-hydroxy-4-methoxybenzophenone (BP-3). The method, validated by use of breast milk pooled samples, shows good reproducibility (inter-day coefficient of variations ranging from 3.5% to 16.3%) and accuracy (spiked recoveries ranging from 84% to 119% at four spiking levels). The detection limits for most of the analytes are below 1 ng mL^{-1} in 100 μL of milk. We tested the usefulness of the method by measuring the concentrations of these twelve compounds in four human milk samples. We detected methyl paraben, propyl paraben, triclosan, BPA, OPP, and BP-3 in some of the samples tested. The free species of these compounds appear to be the most prevalent in milk. Nevertheless, to demonstrate the utility of these measures for exposure and risk assessment purposes, additional data about sampling and storage of the milk, and on the stability of the analytes in milk, are needed.

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1. Introduction

Parabens are esters of *p*-hydroxybenzoic acid widely used as antimicrobial preservatives, particularly against molds and yeast, in cosmetics and pharmaceuticals, and in food and beverage processing [1]. Individually or in combination, parabens are used in over 13,200 formulations [2] in nearly all types of cosmetics. Triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) is a synthetic, broad-spectrum antibacterial agent that has also been used extensively for more than 20 years in a

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variety of consumer products, including toothpaste, mouthwash, deodorants, soaps, textiles (e.g., socks, underwear), and plastic kitchenware [1,3–6]. In Europe, about 350 tons of triclosan are produced annually for commercial applications [7]. In the United States, 76% of 395 commercial soaps examined contained triclosan [6].

Because of the extensive use of parabens and triclosan, human exposure to these compounds is widespread. Specifically, methyl paraben, propyl paraben, and triclosan were detected frequently (>80%) in urine samples collected from the general US population [8,9]. Furthermore, in a recent study, triclosan was detected in human milk from breast milk banks at concentrations ranging from about 100 to 2100 $\mu g\,kg^{-1}$ lipid in 51 of the samples analyzed, although in 11 samples, triclosan concentrations were near or below the detection limit of 5 $\mu g\,kg^{-1}$ lipid [10]. Methyl paraben was also detected in human milk in another study, although the concentration range was not reported [11]. Since breast milk is the main route of exposure to environmental chemicals for breastfed infants, the analysis of breast milk for parabens, triclosan, and other environmental chemicals is of scientific interest.

The analytical technique reported for quantification of such phenols as triclosan or parabens in milk was normally gas chromatography–mass spectrometry (GC–MS) [3,10,12,13]. However, GC methods usually require a relatively large amount of sample (3–10 mL), laborious sample cleanup (e.g., liquid–liquid extraction), and sometimes a derivatization step because of the relatively low volatility of the compounds. We report here the development and validation of an on-line solid-phase extraction-high performance liquid chromatography-tandem mass spectrometry method (on-line SPE-HPLC-MS/MS) with peak focusing to measure the concentrations in milk of five parabens (methyl-, ethyl, propyl-, butyl-, and benzyl parabens), triclosan, and six additional environmental phenols: bisphenol A (BPA); *ortho*-phenylphenol (OPP); 2,4-dichlorophenol (2,4-DCP); 2,5-dichlorophenol (2,5-DCP); 2,4,5-trichlorophenol (2,4,5-TCP); and benzophenone-3 (BP-3).

2. Experimental

2.1. Analytical standards and reagents

Methanol (MeOH) and water, analytical or HPLC grade, were purchased from Caledon (Ontario, Canada). Formic acid (98%) was purchased from EM Science (Gibbstown, NJ, USA). Triclosan; methyl-, ethyl-, propyl-, butyl-, and benzyl parabens; BPA; OPP; 2,4-DCP; 2,5-DCP; 2,4,5-TCP; 4-methylumbelliferyl glucuronide; 4-methylumbelliferyl sulfate; ammonium acetate (>98%); and β-glucuronidase/sulfatase (*Helix pomatia*, H1) were purchased from Sigma–Aldrich Laboratories, Inc. (St. Louis, MO, USA). BP-3 (Eusolex 4360) was provided by EMD Chemicals Inc. (Hawthorne, NY, USA). $^{13}C_{12}$-BPA; $^{13}C_6$-OPP; $^{13}C_6$-2,4-DCP; $^{13}C_6$-2,5-DCP; $^{13}C_6$-2,4,5-TCP; and $^{13}C_4$-4-methylumbelliferone were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). D_3, ^{13}C-BP-3 was obtained from Los Alamos National Laboratory (Los Alamos, NM). $^{13}C_6$-triclosan was purchased from Wellington Laboratories Inc. (Ontario, Canada). D_4-methyl paraben was from CDN Isotopes (Quebec, Canada), and D_4-ethyl-, D_4-propyl-, D_4-butyl parabens were from CanSyn Chem Corp. (Toronto, Canada).

2.2. Preparation of standards and quality control materials

The initial stock solutions of analytical standards and stable isotope-labeled internal standards were prepared by dissolving measured amounts of the analytes of interest in MeOH. Ten working standard spiking solutions, containing all twelve compounds, were generated by serial dilution with MeOH of the initial stock to a final concentration such that a 100-μL spike in 100 μL milk would cover a concentration range from 0.1 to 100 ng mL^{-1} for all of the analytes except triclosan and 2,5-DCP (1–1000 ng mL^{-1}). The stable isotope-labeled internal standard working solution was prepared by diluting the internal standard stock solutions in MeOH, so that a 50-μL aliquot in 100 μL milk resulted in a concentration level of 50 ng mL^{-1}. All standard stock solutions and spiking solutions were dispensed into glass vials and stored at −70 °C until use.

Quality control (QC) materials were prepared from milk purchased from Mother's Milk Bank (San Jose, CA) in 2002 and 2003. The milk was pooled and divided into two aliquots for QC low (QCL) and QC high (QCH) concentration materials. The QCL and the QCH pools were enriched with different levels of native target compounds, mixed thoroughly after preparation, and dispensed in aliquots of 1.5 mL in glass autosampler vials. All QC materials were stored at −70 °C until use. The four milk samples analyzed for the validation of the method were surplus milk that the women had expressed previously and planned to discard. We had no information from the donors or about the sampling procedures. The Centers for Disease Control and Prevention's Human Subjects Institutional Review Board reviewed and approved the study protocol. A waiver of informed consent was requested under 45 CFR 46.116(d). The milk samples were collected in 2007 and stored in 12 mL clear glass vials at −70 °C until analysis.

A mixture of $^{13}C_4$-4-methylumbelliferone, 4-methylumbelliferyl sulfate, and 4-methylumbelliferyl glucuronide was added to all samples to quantify the extent of the deconjugation enzymatic reaction. After incubation, 4-methylumbelliferyl sulfate and 4-methylumbelliferyl glucuronide were hydrolyzed to free 4-methylumbelliferone, and the 4-methylumbelliferone/$^{13}C_4$-4-methylumbelliferone peak area ratio was monitored. The enzyme solution was prepared by dissolving 0.04 g of β-glucuronidase/sulfatase (463 000 U g^{-1} solid) in 10 mL of 1 M ammonium acetate buffer (pH 5.0).

2.3. Sample preparation

Human milk was thawed and vortex-mixed for at least 30 s before aliquoting. For the purpose of estimating the concentrations of total (free + conjugated) species, a 100 μL aliquot of milk was mixed with 50 μL of internal standard solution, 10 μL of $^{13}C_4$-4-methylumbelliferone/4-methylumbelliferyl glucuronide/4-methylumbelliferyl sulfate standard solution (2000 ng mL^{-1} for each compound), and 50 μL of enzyme solution in a disposable 1.5 mL CLIKLOK microcentrifuge tube (Simport, Beloeil, Canada). After gentle

mixing, the sample was incubated at 37 °C for 4 h. Then, 290 μL of MeOH was added to the milk, the sample was vortex-mixed, and it was then centrifuged at 8000 rpm for 15 min. 250 μL of the milk supernatant was then transferred to an autosampler vial, 250 μL of 0.1 M formic acid was added, and the 500 μL sample was vortexed before being placed on the HPLC autosampler for the on-line SPE-HPLC-MS/MS analysis. To determine the concentrations of the free species, we followed the procedures described above, but added 50 μL of 1 M ammonium acetate buffer instead of the enzyme solution and skipped the incubation step. We prepared analytical standards, QCs, and reagent blanks using the same procedure, but we replaced the milk by the same volume of standard stock solution, QC milk or H_2O (for the reagent blank).

2.4. On-line SPE-HPLC-MS/MS

We built the on-line SPE-HPLC-MS/MS system from several Agilent 1100 modules (Agilent Technologies, Wilmington, DE, USA), coupled with an API 4000 Q Trap mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an atmospheric pressure photoionization (APPI) interface. The on-line SPE-HPLC system consisted of two binary pumps with degassers, an autosampler with a 900-μL injection loop, a high-pressure mixing Tee, and one column compartment with a 10-port switching valve. The mass spectrometer and Agilent modules were programmed and controlled by use of Analyst 1.4.1 software (Applied Biosystems), and the on line SPE-HPLC-MS/MS acquisition method was built in 'LC sync' mode. The SPE column was a LiChrosphere RP-18 ADS (25 mm × 4 mm, 25 μm particle size, 60 Å pore size, Merck KGaa, Germany), and the HPLC column was an Agilent Zorbax Eclipse XDB-C8 (150 mm × 4.6 mm, 5 μm).

The on-line SPE-HPLC-MS/MS system used in the current study is a modified version of the one we used for measuring eight environmental phenols in milk previously [14]. We replaced the six-port switching valve with a 10-port valve, so that the system could perform peak focusing of the analytes (Fig. 1). The procedure for extracting the parabens, triclosan, and the other phenols from the milk involved three periods. During the first period (0–3 min), with the 10-port valve at position 1–2, 500 μL of the sample injected was loaded onto the SPE column by the SPE pump with 20% MeOH: 80% H_2O at a flow rate of 1 mL min^{-1}. During the second period (3–5 min), the 10-port valve switched to its alternate position (1–10), and the analytes retained on the SPE column were backeluted by the HPLC pump, with 50% MeOH:50% H_2O at a flow rate of 0.5 mL min^{-1}. At the same time, the SPE eluate was diluted through a mixing tee for peak focusing of the analytes, with 80% H_2O at a flow rate of 0.25 mL min^{-1} provided by the SPE pump. During the third period (5–15 min), the 10-port valve switched back to its original position (1–2), and the analytes were transferred to the HPLC column by the HPLC pump at a flow rate of 0.75 mL min^{-1}, using the following gradient program: 5.1–13 min, 70% MeOH to 90% MeOH; 13.1–14 min, 100% MeOH; 14.1–15 min, 50% MeOH. Regeneration of the SPE column with 100% MeOH from 5 to 10 min and SPE column equilibration with 20% MeOH:80% H_2O from 10 to 15 min at a flow rate of 1 mL min^{-1} were also performed during this third period.

2.5. Mass spectrometry

The analytes in the milk were ionized by the process of photoionization, induced by a continuous beam of ultraviolet radiation in the presence of a dopant (toluene) within the source housing. The dopant was provided at a flow rate of 75 μL min^{-1} by an isocratic pump controlled by use of the Analyst 1.4.1 software. The APPI negative ion mode settings used were curtain gas (N_2) flow: 20 arbitrary units (au), collision gas (N_2) flow: 9 au, nebulizer gas (N_2) flow: 60 au, lamp gas (N_2) flow: 20 au, nebulizing gas temperature: 500 °C, and ion transfer voltage: −800 V. Q1 and Q3 were set at unit resolution. Ionization parameters and collision cell parameters were optimized separately for each analyte. The negative fragment ions used for quantification and confirmation and the retention times of the analytes are listed in Table 1.

3. Results and discussion

3.1. Sample pretreatment

To maximize the lifetime of the SPE and HPLC columns, we centrifuged the milk and diluted the supernatant with 0.1 M formic acid before the sample was injected onto the on-line SPE-HPLC-MS/MS system. Since some of the analytes included in the current method were lipophilic, such as triclosan ($\log K_{ow} - 4.76$) and BP-3 ($\log K_{ow} - 3.79$), we initially added 190 μL of 2-propanol to 100 μL of milk before the centrifugation step to dissolve the lipids and achieve a better recovery of these analytes during centrifugation, as we had done before [14]. However, because 2-propanol interfered with

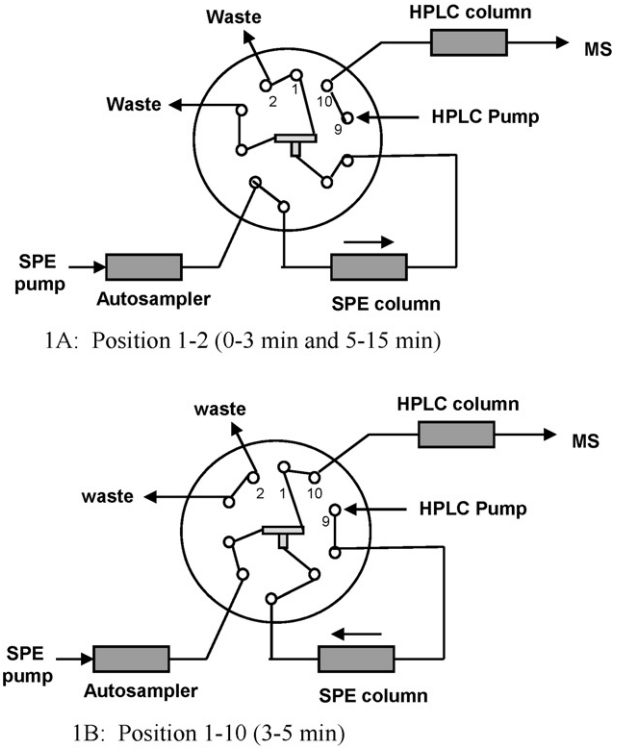

Fig. 1 – On-line SPE-HPLC-MS/MS setup.

Table 1 – Analyte retention time (RT), and precursor ion → product ion transitions monitored for quantitation (and confirmation) of native compounds and corresponding isotope-labeled internal standards

Analyte	RT (min)	Precursor ion → product ion (m/z)	
		Native analyte	Internal standard
Methyl paraben	8.0	151 → 92 (136)	155 → 96
Ethyl paraben	8.5	165 → 92 (137)	169 → 96
Propyl paraben	9.2	179 → 92 (136)	183 → 96
Butyl paraben	10.1	193 → 92 (136)	197 → 96
Benzyl paraben	10.0	227 → 92 (136)	197 → 96[a]
Triclosan	13.2	252 → 216 (287 → 142)	264 → 228
Bisphenol A	9.2	227 → 133 (212)	239 → 139
Benzophenone-3	11.3	227 → 183 (211)	231 → 183
2,4-Dichlorophenol	6.8	161 → 125 (163 → 125)	167 → 131
2,5-Dichlorophenol	10.0	161 → 125 (163 → 125)	167 → 131
2,4,5-Trichlorophenol	11.3	195 → 159 (197 → 161)	201 → 165
Ortho-phenylphenol	10.0	169 → 115 (141)	175 → 121

[a] D_4-butyl paraben is used as the internal standard of benzyl paraben.

the measurement of methyl paraben, we used MeOH instead. As we expected, we also found that the recovery of these lipophilic analytes, specifically triclosan and BP-3, improved with increasing amounts of MeOH. However, too much MeOH could facilitate the breakthrough of the more polar compounds (e.g., parabens and BPA) during SPE. We found that adding 290 μL of MeOH for every 100 μL of milk provided the best recovery of triclosan and BP-3 without compromising the SPE recovery of parabens and BPA. After centrifugation, we also added formic acid to the supernatant to facilitate the retention of the more acidic chlorophenols, specifically 2,4,5-TCP, to the SPE sorbent [14].

3.2. *Peak focusing of the analytes*

One of the most common limitations of on-line SPE is compromised HPLC resolution [15]. In our method, elution of the phenols of interest from the SPE column required 0.5 mL min^{-1} 50% MeOH:50% water for 2 min. However, a starting HPLC gradient with 50% MeOH content would broaden the chromatographic signals of the most polar phenols (e.g., methyl paraben). Using a 10-port switching valve and a mixing Tee, we diluted the HPLC flow (0.5 mL min^{-1} 50% MeOH) with 80% H_2O (0.25 mL min^{-1}) provided by the SPE pump, and the peak shapes of the parabens, especially methyl paraben, improved greatly (i.e., the peak width at half height decreased from 0.25 to 0.12 min).

In our previous method, we used a pair of Chromolith™ Performance RP-18 (100 mm × 4.6 mm) HPLC columns for the chromatographic separation of the analytes [14]. For the current method, we selected a Zorbax Eclipse XDB-C8 (150 mm × 4.6 mm, 5 μm) HPLC column. With this column and under the present experimental conditions, the run time was 15 min, 9 min shorter than before [14], even though we included five parabens and triclosan in the current method. This greatly improved the overall throughput of the analysis. Nevertheless, the baseline separation of the two isomers 2,4-DCP and 2,5-DCP was still achieved, due, at least in part, to the excellent peak shapes of the 2,4-DCP and 2,5-DCP signals, as demonstrated in Fig. 2.

3.3. *MS detection*

Because of the poor ionization of triclosan under the conditions required for atmospheric pressure chemical ionization

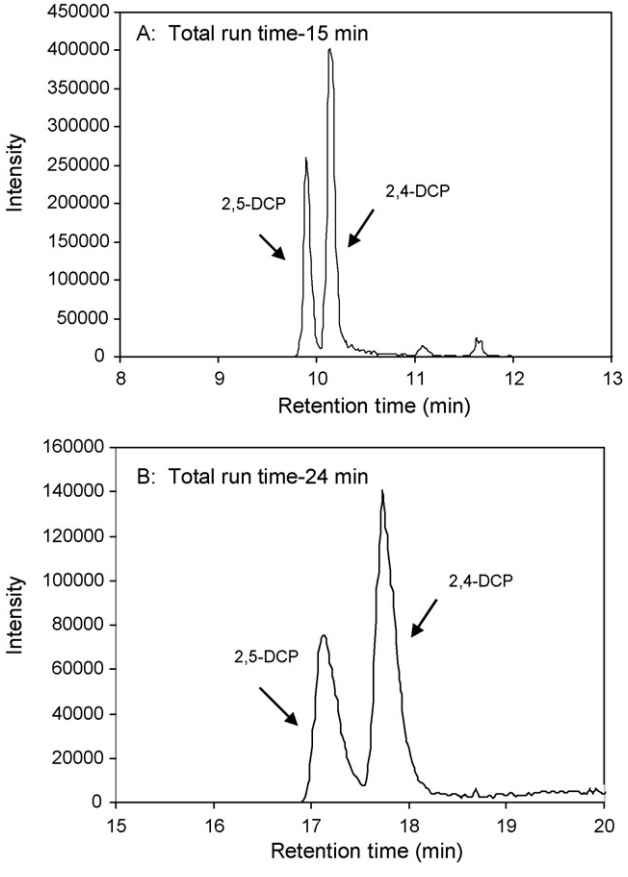

Fig. 2 – Separation of 2,4-DCP and 2,5-DCP isomers from the extracted multiple reaction monitoring ion chromatograms using a (A) Zorbax Eclipse XDB-C8 as HPLC column (current method); (B) pair of Chromolith™ Performance RP-18 as HPLC columns (previous method) [14]. The SPE and HPLC gradient programs were different for the two methods.

(APCI), we chose a different ionization technique, APPI, for the current method. The full scan mass spectrum of triclosan by APPI gave three ions at *m/z* 287 $[M-H]^-$, *m/z* 252 $[(M-H)-Cl]^-$, and *m/z* 161 (dichlorophenol ion from the breakdown of triclosan in Q_0). The product ion scan of *m/z* 252 gave an ion at *m/z* 216. More importantly, the APPI transition *m/z* 252 → 216 was about ten times more sensitive than *m/z* 287 → 142 and 161 → 125, the ion transitions used to quantify triclosan in urine by APCI [16]. Therefore, for triclosan, we monitored two APPI transitions: *m/z* 252 → 216 (quantitation ion) and 287 → 142 (confirmation ion). The use of APPI also improved the ionization of OPP and BP-3 (data not shown). For the rest of the analytes, ionization efficiency was comparable regardless of the technique used.

3.4. *Method validation and quality control*

Milk spiked with standard and isotope-labeled standard solutions was analyzed repeatedly to determine the LODs, accuracy, and precision of the method. The LODs were calculated as $3S_0$, where S_0 is the standard deviation as the concentration approaches zero [17]. S_0 was determined from five repeated measurements of three low-level standards prepared in milk. The calculated method LODs ranged from 0.1 to 0.4 ng mL^{-1} (Table 2), except for triclosan (1.0 ng mL^{-1}). These values reflect the good sensitivity of the method, especially considering the relatively low sample volume (100 μL) used. The method accuracy was assessed by five replicate analyses of milk spiked at four different concentrations and was expressed as the percentage of expected levels (Table 2). The intraday variability, reflected in the method accuracy, ranged from 84% to 119% for all of the analytes at the four spike levels (Table 2). We determined the method precision by using 40 repeated measurements of QCL and QCH materials over a period of two weeks (Table 2). The R.S.D.s, which reflect the intra- and inter-day variability of the method, ranged from 3.5% to 16.3%. Typical chromatograms of a milk blank and a milk blank spiked with a low concentration standard are shown in Fig. 3.

We obtained calibration curves after spiking water or milk with the analytical standards. Because the slopes of the calibration curves from water and from milk were very similar (data not shown), for quantification we used the calibration curve obtained from spiking water. Calibration curves in water showed adequate linearity, up to 100 ng mL^{-1} for all of the analytes except for triclosan and 2,5-DCP (up to 1000 ng mL^{-1}), with correlation coefficients greater than 0.99. Interday variation of the calibration curve slopes, measured as the RSD, was less than 10%.

The SPE recoveries of the analytes from milk were calculated as described before [14]. In general, we obtained very good SPE recoveries (75–105%) for all of the analytes (Table 2), except for triclosan (62%). Although the SPE recovery for triclosan is slightly lower than desired, the sensitivity (LOD = 1.0 ng mL^{-1}) and accuracy (spiked recoveries are between 90% and 104% at four spiking levels) are still acceptable (Table 2), attributed to the use of the isotope-labeled triclosan as an internal standard.

Table 2 – Solid-phase extraction (SPE) recoveries, method accuracy, limits of detection (LODs), and method precision

Analyte	SPE Recovery (%)	Spiked recovery (%)[a]			LOD (ng mL^{-1})	QC low		QC high	
						Mean (ng mL^{-1})	R.S.D. (%)	Mean (ng mL^{-1})	R.S.D. (%)
Methyl paraben	95	(0.5) 90	(1) 109	(5) 109	(10) 103	0.1	5.6	5.3	22.1	3.5
Ethyl paraben	99	(0.5) 107	(1) 108	(5) 102	(10) 105	0.1	5.2	5.4	21.2	5.6
Propyl paraben	103	(0.5) 106	(1) 103	(5) 102	(10) 105	0.1	5.3	5.1	21.2	4.0
Butyl paraben	89	(0.5) 119	(1) 111	(5) 102	(10) 106	0.1	5.0	4.5	20.3	4.7
Benzyl paraben	87	(0.5) 106	(1) 105	(5) 93	(10) 99	0.1	4.9	7.7	19.7	6.0
Triclosan	62	(5) 97	(10) 90	(25) 104	(50) 97	1.0	10.2	16.3	19.2	11.0
Bisphenol A	105	(0.5) 90	(1) 109	(5) 109	(10) 103	0.3	9.3	8.3	22.7	6.3
Benzophenone-3	102	(1) 107	(5) 98	(10) 107	(25) 101	0.4	4.5	12.0	18.7	8.8
2,4-Dichlorophenol	88	(0.5) 102	(1) 98	(5) 84	(10) 90	0.1	3.8	9.7	15.3	8.0
2,5-Dichlorophenol	91	(1) 105	(5) 107	(10) 103	(50) 105	0.4	4.7	9.8	19.6	8.6
2,4,5-Trichlorophenol	75	(0.5) 103	(1) 103	(5) 105	(10) 106	0.1	4.3	9.6	17.5	7.8
Ortho-phenylphenol	88	(0.5) 92	(1) 109	(5) 103	(10) 100	0.3	4.3	7.7	17.7	7.0

The values in italic (in parenthesis) were concentrations of standards which were used to calculate SPE recovery.

[a] *Note:* The concentrations of spiked standards were in parenthesis.

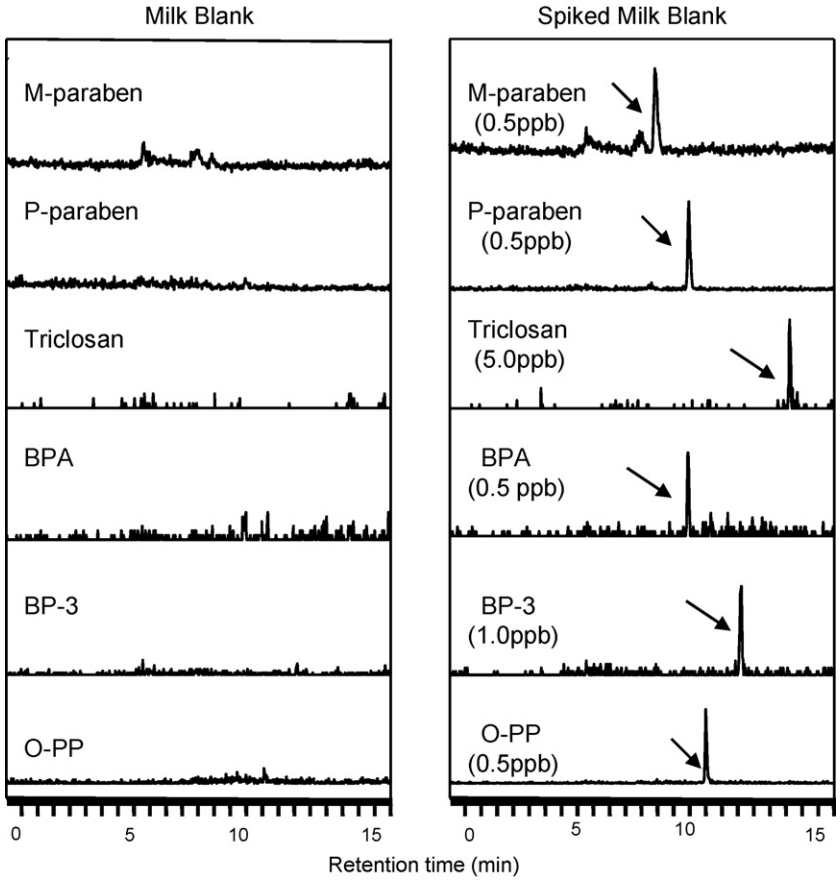

Fig. 3 – Typical HPLC-MS/MS extracted ion chromatograms for a milk blank (left) and a milk blank spiked with a low concentration calibration standard (right). For illustration purposes, the y-axis scales of the milk blank were magnified 5× to 10× times compared to those used for displaying the chromatograms of the spiked milk. Some analytes were not included in the chromatograms because of the low frequency of detection in the milk.

Table 3 – The concentrations of free and total (free plus conjugated) species of selected parabens and environmental phenols in 4 human milk samples[a]

Compound	Milk 1 conc. (ng mL^{-1})	Milk 2 conc. (ng mL^{-1})	Milk 3 conc. (ng mL^{-1})	Milk 4 conc. (ng mL^{-1})
Methyl paraben free	<LOD	0.32	3.04	<LOD
Methyl paraben total	0.53	0.70	3.00	0.73
Propyl paraben free	<LOD	<LOD	0.32	<LOD
Propyl paraben total	<LOD	<LOD	0.33	<LOD
Triclosan free	2.81	<LOD	13.8	<LOD
Triclosan total	3.39	<LOD	14.5	<LOD
BPA free	0.45	0.79	1.54	0.41
BPA total	0.91	0.80	1.62	0.73
OPP free	<LOD	<LOD	0.12	<LOD
OPP total	<LOD	<LOD	0.19	<LOD
BP-3 free	<LOD	1.24	<LOD	<LOD
BP-3 total	<LOD	1.28	<LOD	<LOD

The limits of detection (LODs) were 0.1 ng mL^{-1} (methyl and propyl paraben), 0.3 ng mL^{-1} (BPA, OPP), 0.4 ng mL^{-1} (BP-3), and 1.0 ng mL^{-1} (triclosan).

[a] *Note*: The concentrations of the other analytes were <LOD in all samples analyzed.

3.5. *Quantification of parabens, triclosan, and phenols in milk samples*

We applied our method to determine free and total (free plus conjugated) concentrations of five parabens, triclosan, and the other six environmental phenols in milk samples collected in 2007 from four anonymous lactating women with no known occupational exposure to these compounds. We detected methyl paraben, propyl paraben, and triclosan in some of these samples (Table 3). The total concentrations of triclosan in these samples ranged from below LOD (1 ng mL^{-1}) to 14.5 ng mL^{-1}. Assuming an approximate 4% lipid con-

tent in milk as suggested before [18,19], the concentrations of triclosan found in these samples ranged from <LOD to 345 $\mu g\,kg^{-1}$ lipid, similar to the concentrations reported before [10]. We also detected BPA, BP-3, and OPP in some of the samples tested (Table 3); the presence of these compounds in milk has been reported before [14,20]. In agreement with our previous findings [14], we noted that, when detected, the concentrations of the free species were very similar to the concentrations of total species. These data suggested that the free species, rather than the corresponding conjugates, may prevail in human milk. However, since we had no information regarding the collection and storage of these four samples, we cannot rule out the potential for contamination with these ubiquitous compounds during collection or storage. Therefore, additional studies are needed to determine the degree of conjugation of environmental chemicals in milk, including phenols and parabens.

4. Conclusions

We developed a sensitive, selective, and precise automated on-line SPE-HPLC-MS/MS method with peak focusing for the simultaneous quantitative measurement of five parabens, triclosan, and six environmental phenols in milk. The method required a small amount of milk (0.1 mL) and minimum sample preparation. This method is rugged and labor- and cost-effective, while it also allows for the analysis of a large number of samples for epidemiological studies. However, to demonstrate the utility of these measures for exposure and risk assessment purposes, additional information about collection, handling, and storage of the samples, as well as data on the stability of the analytes in milk, are needed.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.aca.2008.05.068.

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