



## Validation of analytical methodology for determination of Personal Care Products in environmental matrix by GC-MS/MS

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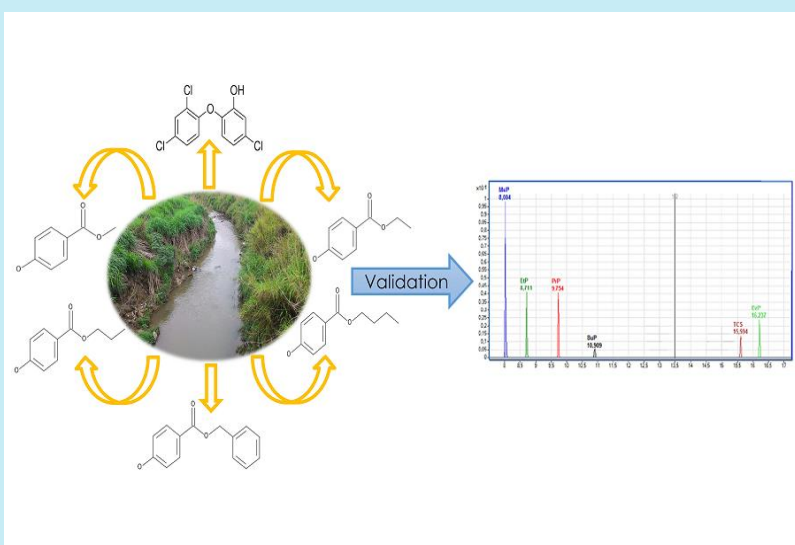
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**ABSTRACT:** The presence of personal care products in the environment is recent and few researches work with the quantification of this class of emerging contaminants in Brazil variety of these products is released into the aquatic environment. The growing interest in these substances occurs mainly because they exhibit biological activity in very low concentrations, which gives a great environmental relevance. The present study aims to validate a methodology and verify its efficiency in the determination of six personal care products, among them parabens and triclosan. The samples were submitted to the solid phase extraction process and were later analyzed by gas chromatography coupled with mass spectrometry for the determination of personal care products. The validation of the methodology used was based on the standards established by the National Health Surveillance Agency. The method showed good recoveries (56 - 117%) and the limits of detection of the method ranged between 0.9 and 14.6 ng L<sup>-1</sup>, while the limits of quantification were within the 3.1- 48.7 ng L<sup>-1</sup>. Reproducibility and repeatability, expressed as coefficient of variation, had satisfactory values (<15%). The extraction and quantification method were efficient for the determination of these analytes in water samples.



### 1. Introduction

Personal care products (PCPs) are used at scale and their presence in aquatic environments has received increasing attention from the scientific community with recent studies indicating the toxic potential to the environment<sup>1-3</sup>.

PCPs are present in UV filters (benzophenones), preservatives (parabens), antimicrobials (triclosan), fragrances, repellents

among others. One of the most studied PCPs and among the ten organic compounds normally detected in water is triclosan<sup>4,5</sup>. It has been used in a large number of PCPs, and can be found in soaps, deodorants, body moisturizers, toothpastes and also a component of polymers and fibers<sup>6</sup>. Parabens also comprise a group that is currently under study, most are part of the formulation of various cosmetics and are also used as preservatives in the food industry<sup>7</sup>.

As for the instrumental analytical technique, the most used for the determination of parabens and other classes of contaminants in waters is the chromatography, being able to be used both gas and liquid, both of which can be coupled to different types of detectors to obtain methods even more sensitive and selective<sup>8</sup>. Another analytical method that is being used is the capillary electrophoresis, due to the low cost and the possibility to determine the concentration of the compound of interest directly in the sample, without pre-treatments or previous separations<sup>9</sup>.

The concern of the scientific community with the damages that these contaminants can cause, especially in aquatic environments, the current research has been aimed at implementing and validating new analytical methods that are more sensitive and precise, allowing the advancement of research related to the evaluation of the quality of water resources in terms of micropollutants<sup>10-12</sup>.

The aim of this study was validated an analytical methodology applied in the determination of PCPs in surface water by gas chromatography coupled to mass spectrometry (GC-MS/MS).

## 2. Experimental

### 2.1 Reagents, solvents and analytical standards

All analytical standards (MeP (99%), EtP (99%), PrP (99%), butylparaben (BuP, 99%), triclosan (97%), N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS, 98.5%) and solvents were purchased from Sigma Aldrich (Steinheim, Germany). The SPE C18 6 mL cartridges with 1000 mg of adsorbent phase were provided from Hexis (Jundiaí, SP). The solvents used in sample preparation and chromatography analysis (methanol, hexane, ethyl acetate, acetonitrile, and acetone), were HPLC-grade, from Sigma Aldrich. Merck (Darmstadt, Germany) supplied hydrochloric acid (HCl, analytical grade) and porosity membranes of cellulose acetate were acquired from Millipore (Billerica, MA). Ultrapure water was prepared using a water purification system from Millipore. N<sub>2</sub> and He gases (purchased

from White Martins, Rio de Janeiro, Brazil) were 5.0 purity grade.

### 2.2 Extraction

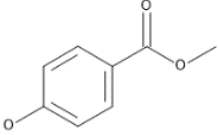
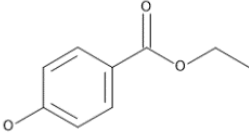
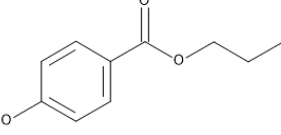
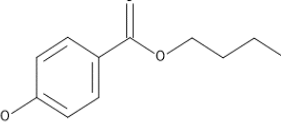
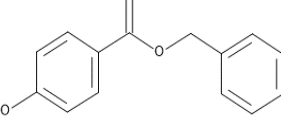
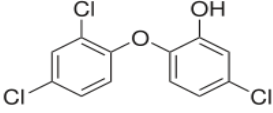
The PCPs analyzed in this work are present in Table 1, with their respective classes. These compounds were chosen because of the large production and the wide consumption by the population in general.

In order to apply the methodology to the real matrices, samples were collected at seven points in the Palmital river basin, located in the state of Paraná, specifically in the Metropolitan Region of Curitiba (MRC). Surface water samples were collected in four campaigns performed in October/2016, February/2017, May/2017 and July/2017.

The methodology used for the extraction of PCPs was adapted from Ide, Cardoso e Marques (2013)<sup>13</sup>. First, 1 liter of sample was filtered on 0.45 µm cellulose acetate membranes to remove particulate matter. Then the pH of the sample was adjusted to 3 with addition of HCl 6 mol L<sup>-1</sup>. For the solid phase extraction (SPE), C18 (Agila SampliQ 1000 mg - C18 6 mL) cartridges were preconditioned with 6 mL of hexane, 6 mL of acetone, 6 mL of methanol and 6 mL of ultra-water-pure. Samples were run through the cartridges in a continuous stream and then dried for 30 min. Elution of the analytes was done using 6 mL of acetonitrile and 6 mL of acetone, collected in round bottom flasks. The samples were taken to dry in a rotary evaporator and then reconstituted with 1 mL of acetonitrile and then subjected to ultrasound equipment.

Following the procedures mentioned above, 200 µL of the extracted sample was separated for the derivatization process for further analysis by gas chromatography. To carry out the derivatization process, the samples were first evaporated in 350 µL inserts in a 40 °C oven, after being completely evaporated, 50 µL of the derivatization agent (BSTFA + 1% TMCS) was added at a temperature of 60 °C for 30 min for the reaction. After this step, 150 µL of ethyl acetate was added for sample reconstitution.

**Table 1.** Compounds studied

Compound	Structures	Acronym	Class	<sup>a</sup> log K <sub>ow</sub>
Methylparaben		MeP	Preservative	1.96
Ethylparaben		EtP	Preservative	2.47
Propylparaben		PrP	Preservative	3.04
Butylparaben		BuP	Preservative	3.57
Benzylparaben		BzP	Preservative	3.59
Triclosan		TCS	Antibacterial	4.76

<sup>a</sup>log K<sub>ow</sub>: Octanol/water partition coefficient. Source: TOXNET, 2017<sup>11</sup>.

### 2.3 Chromatographic Analysis

The compounds were analyzed on a 7890A GC-MS/MS (Agilent Technologies), equipped with a HP-5msi (30 m, 0.25 mm, 0.25 μm) silica capillary column coupled to a triple mass spectrometer quadrupole model 7000 with automatic sampler (PAL Sampler).

The chromatographic conditions applied were based on the method proposed by Mizukawa (2018)<sup>14</sup>. The injection was performed in Splitless mode. The temperature of the injection door was 280 °C and 2 μL of each sample were injected. A constant flow of 1 mL min<sup>-1</sup> of helium was used as carrier gas. The temperature of the oven was set at 100 °C (maintaining this temperature during 2 min), followed by an elevation of 15 °C min<sup>-1</sup> until 180 °C, 6 °C min<sup>-1</sup> until 270 °C, and 5 °C min<sup>-1</sup> until 310 °C, maintaining this temperature during 3 min. The resulting run time was 33.33 min. Temperatures of the transference

line and the ionization source were 280 °C. Nitrogen was used as a collision gas in a flux of 1.5 mL min<sup>-1</sup>. Detection and quantification by MS/MS were performed in monitoring reaction mode.

### 2.4 Validation of the Chromatographic Method

In this study, the following parameters were evaluated: Linearity, sensitivity, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision.

The methodology used to validate the analytical method was based on ANVISA Resolutions 475/02<sup>15</sup> and 899/03<sup>16</sup> and INMETRO's DOQ-CGCRE-008/03<sup>17</sup>.

#### 2.4.1 Linearity and Sensitivity

An external standardization was used for the quantification of the compounds. Individual

standard stock solutions were prepared in methanol with a concentration of 1000 mg L<sup>-1</sup> and stored in a freezer. The mixed stock solutions were prepared diluting the standard individual stock solutions. Working solutions for the calibration solutions were prepared by direct dilution of the 10 mg L<sup>-1</sup> mix. Linearity was obtained by constructing an analytical curve with at least five concentrations, ranged between 0.05 – 1.0 mg L<sup>-1</sup>. The sensitivity was expressed by the slope of the linear regression analytical curve and determined simultaneously with the linearity tests.

#### 2.4.2 Limits of Detection and Quantification

LOD and LOQ were determined by Equations 1 and 2:

$$LOD = \frac{3 S_a}{IC} \quad (1)$$

$$LOQ = \frac{10 S_a}{IC} \quad (2)$$

where  $S_a$  is the estimate of the standard deviation of at least three whites and  $IC$  is the slope of the analytical curve.

#### 2.4.3 Accuracy

Accuracy was assessed by ultrapure water recovery test. The value can be estimated by Equation 3:

$$\%recovery = \frac{x_{got}}{x_{added}} \times 100 \quad (3)$$

#### 2.4.4 Precision

Precision was evaluated in terms of repeatability and intermediate precision by

calculating the absolute standard deviation and coefficient of variation for a minimum of six replicates.

### 3. Results and Discussion

The quality control parameters for the compounds analyzed are present in Table 1. The linearity of the method was measured by the linear regression coefficient and all the analytical curves had a minimum correlation coefficient equal to 0.99, so they are in accordance with what is recommended by ANVISA resolution 899/03<sup>16</sup>.

The sensitivity was expressed by the angle coefficient of the calibration curve that is, by the slope of the analytical curve and through it was possible to verify the tendencies of the sensitivity for each analyte. It is observed that propylparaben (PrP) was the compound that presented the highest sensitivity among the six personal care products analyzed by this analytical method. However, the sensitivity is not directly related to low detection limit values, but with a better precision in the quantification of values with similar concentrations<sup>18</sup>.

The lowest LOD and LOQ were found for benzylparaben (0.5 and 1.6 ng L<sup>-1</sup>, respectively) and the highest for Methylparaben (14 and 48 ng L<sup>-1</sup>). The limits of quantification were comparable to other studies that used the same type of detection and were satisfactory.

The accuracy of the proposed method was obtained from the analyte recovery test, which determines the recovery of the solid phase extraction by means of known concentrations of the compounds (0.1, 0.2, 0.4, 0.8 and 1.0 µg L<sup>-1</sup>). Table 2 shows the mean values obtained in the recovery test of the compounds worked.

**Table 2.** Linear range, Analytical Curve, Correlation Coefficient ( $R^2$ ), Sensitivity (slope), Method Limits of Detection and Quantification for Selected Compounds and Recovery Rates (R%).

Compound	Linear range (ng L <sup>-1</sup> )	Analytical curve	$R^2$	Slope	LOD (ng L <sup>-1</sup> )	LOQ (ng L <sup>-1</sup> )	Recovery Rates (%)
MeP	50 – 1000	$y = 174225x - 1617$	0.9956	$1.7 \times 10^5$	14	48	56.8
EtP	50 – 1000	$y = 158440x - 3018$	0.9972	$1.5 \times 10^5$	3.2	10	78.5
PrP	50 – 1000	$y = 203131x - 4872$	0.9981	$2.0 \times 10^5$	0.9	3.2	111.4
BuP	50 – 1000	$y = 141603x - 3667$	0.9984	$1.4 \times 10^5$	6.9	23	113.5
BzP	50 – 1000	$y = 126372x - 4773$	0.9943	$1.2 \times 10^5$	0.5	1.6	117.9
TCS	50 – 1000	$y = 79002x - 2086$	0.9984	$7.9 \times 10^4$	7.9	26	114

The method proved to be efficient for most of the compounds analyzed. With the exception of MeP and EtP, all other compounds recovered 100%. The low recovery rates of MeP and EtP can be justified by their low log  $K_{ow}$  values (1.96 and 2.47 respectively). According to the literature, the more polar the compounds ( $\log K_{ow} \leq 3$ ), the efficiency of SPE is usually lower due to the solid phase used, octadecylsilane, that has a non-polar character<sup>19</sup>.

The results concerning repeatability and reproducibility are present, respectively, in Table 3. ANVISA (2003)<sup>16</sup> does not admit, in precision analysis, coefficient of variance (CV) values higher than 5% for detection of drugs in pharmaceutical products, but for complex matrices this value does

not apply. In Resolution 475/02 of ANVISA (2002)<sup>14</sup> it is stated that for more complex samples (blood, serum or plasma) CV values of up to 15% are allowed. In turn, INMETRO (2003)<sup>17</sup> allows a CV of up to 20% for precision analysis of the method. In this work, the mean values of CV for analysis of repeatability and intermediate precision are within the established limit (15% - 20%), and therefore the method can be considered accurate for the analysis of the five PCPs. For EtP only, the reproducibility at the lowest concentration was above 15%. The best result among the PCPs studied was for the BzP, with average values 1.3% in intraday and 7.5% in between subsequent days.

**Table 3.** Coefficient of variance expressed as percentage of the intraday and between subsequent days assay of the compounds studied at three different concentrations (n=5)

Analitos	Repeatability				Reproducibility			
	0.05 mg L <sup>-1</sup>	0.2 mg L <sup>-1</sup>	1.0 mg L <sup>-1</sup>	Average	0.05 mg L <sup>-1</sup>	0.2 mg L <sup>-1</sup>	1.0 mg L <sup>-1</sup>	Average
MeP	2.8	1.1	4.6	2.8	10.3	5.1	10.8	8.7
EtP	1.4	2.5	2.0	2.0	18.0	2.9	6.1	9.0
PrP	2.7	6.0	4.9	4.5	14.9	10.5	8.6	11.3
BuP	0.9	1.2	4.4	2.2	15.7	7.0	3.6	8.8
BzP	1.2	1.4	1.2	1.3	7.7	5.6	9.2	7.5
TCS	1.9	1.7	0.5	1.4	10.4	9.6	9.8	9.9

### 3.1 Determination of parabens and triclosan in surface waters of the Palmital River

With the determination and evaluation of the main validation parameters, it was possible to apply the proposed chromatographic method in the analysis of PCPs in samples collected in the Palmital River.

In every sample analyzed in this research at least one of the parabens and triclosan were detected. Table 4 shows the concentration range found during the four sampling campaigns in the Palmital River. MeP and PrP were the parabens detected with the highest concentrations during the sampling campaign, reaching 0.40 and 0.22  $\mu\text{g L}^{-1}$ . Differently from the parabens, the TCS was determined with greater frequency and in greater concentrations in the campaign of October of 2016, being that the greater concentration of this compound was of 0.19  $\mu\text{g L}^{-1}$ .

From the results quoted above it was verified that the method was efficient to quantify the low concentrations detected in the Palmital River.

**Table 4.** Concentration range and average concentration of PCBs on the Palmital river.

Compound	Concentration ( $\mu\text{g L}^{-1}$ )	Average conc. ( $\mu\text{g L}^{-1}$ )
MeT	0.05 – 0.40	0.07
EtP	0.05	0.01
PrP	0.004 – 0.22	0.04
BuP	0.02 – 0.04	0.01
BzP	0.003 – 0.13	0.04
TCS	0.03 – 0.21	0.06

## 4. Conclusions

The results obtained in the validation of the chromatographic method were satisfactory and provided its reliability. All the parameters of merit worked had results according to the norms used for validation of analytical methods. The limits of detection and quantification were satisfactory and allowing the quantification of analytes at traces levels, in the  $\text{ng L}^{-1}$  range. With the application of the methodology, all the compounds studied were



quantified in the Palmital River, at least once at in trace levels.

The proposed methodology applies perfectly in the purpose initially established the detection of PCPs in surface waters and, finally, this method can be used as a basis for future monitoring of these compounds in environmental samples.

## 5. Acknowledgements

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