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ORIGINAL PAPER

Detection of Steroids in Tap and Drinking Water Using an Optimized Analytical Method by Gas Chromatography–Mass Spectrometry

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Abstract Endocrine-disrupting chemicals can produce effects on the human health or living beings. Hence, it is of high importance to determine their presence in water. This work presents a reliable method for determining 17β-Estradiol (E_2) and 17α-Ethinylestradiol (EE_2) in tap and drinking water. The analytic method proposed was optimized by spiking ultrapure water samples with a known amount of steroids in terms of solid phase extraction by varying elution solvent volume and analyte mass in the cartridge, the extract concentration by using either distinct temperatures in rotary evaporator or nitrogen gentle stream, and the solvent effect in chemical derivatization with *N*,*O*-bis (trimethylsilyl) trifluoroacetamide:trimethylchlorosilane (1%). The performance of the analytical method was assessed and applied to real samples; the efficiency of extraction and derivatization procedure ranged from 81 to 100% for E_2 (CV 4–19%) and from 82 to 96% for EE_2 (CV 4–18%). Limits of detection (quantification) were 1.0 (3.0) ng/L and 3.0 (10.0) ng/L for E_2 and EE_2, respectively. Analysis of the drinking water samples yielded concentrations ranging from 3.0 to 11.4 ng/L for E_2 and from 10.0 to 246 ng/L for EE_2. Analyses of steroids in tap water were found below the limit of detection. Consumption of drinking water in the presence of endocrine-disrupting chemicals could be a risk for the users in the long term and their consumption should be avoided under the principle of prevention.

Keywords Endocrine disrupters · Drinking and tap water · Steroids · Gas chromatography–mass spectrometry

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Introduction

Emerging contaminants (ECs) are chemical compounds present in the environment, in particular in water bodies at concentrations as low as ng/L and μg/L. Those compounds have been recently classified as a potential environmental factor affecting the metabolic processes of living organisms, including humans (Barceló and Petrovic 2008). Steroids are ECs, classified as endocrine disruptor compounds with a negative impact on the hormonal balance of animals and humans. 17β-Estradiol (E_2) and 17α-Ethinylestradiol (EE_2) are the steroids most frequently identified in the environment due to its wide consumption (Kandarakis et al. 2009). E_2 is a natural hormone, relatively bioaccumulative and persistent in the environment, while EE_2 is a synthetic hormone obtained from cholesterol and is the active ingredient in birth control pills (Velicu and Suri 2009). Steroid effects in living beings have been analyzed in various studies. Male fish were exposed at 4 ng/L of EE_2 altering their sex ratio and the secondary sexual characteristics (Länge et al. 2001). Also the estrogenic activity using the vitellogenin induction in fathead minnows was analyzed, where a response of the test was

found between 32 and 100 ng/L of E_2 and a response between 0.1 and 1 ng/L of EE_2 after a 3-week exposure (Brian et al. 2005). E_2 and EE_2 are hydrophobic organic compounds (low solubility in water) and it can be seen in their physicochemical properties (Table 1).

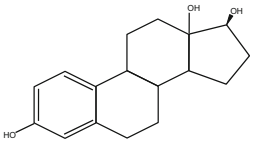
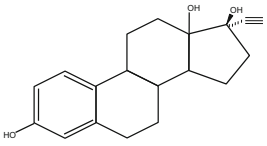
The water quality impacts on public health and ecosystems and frequently it is associated with acute and chronic diseases, either by direct ingestion or through contamination of food and water (Bergman et al. 2013). Currently, the world is increasing the processing, marketing, and consumption of bottled water, mainly due to mistrust of the quality of local water supply systems. In terms of value, the total global bottled water market for 2010 was estimated at approximately 82 billion US dollars (Rani et al. 2012). On the other hand, the occurrence of ECs in sources of drinking water such as rivers, lakes, lagoons, or well would have potential impacts on human health and living beings. Steroids were detected in surface water downstream from the wastewater treatment plants (WWTP) at concentrations ranging from 1 to 75 ng/L for E_2 in the United States (Velicu and Suri 2009) and from 0.2 to 7 ng/L for EE_2 from effluents of WWTP in the United Kingdom (Desbrow et al. 1998). In surface water, EE_2 was detected at 11.1 ng/L in the United States (Zuo et al. 2013); E_2 was found at 15 ng/L in Taiwan; E_2 and EE_2 were found at 6.6 and 2.2 ng/L, respectively, in Spain (Gorga et al. 2015); and in spring water E_2 and EE_2 were found at 0.02 and 0.06 ng/L, respectively, in Mexico (Gibson et al. 2007). There is limited information on the presence of steroids in local tap water supply systems of the cities in the world (Chia-Yang et al. 2007).

The ECs have been detected in groundwater sites that are drinking water source in the United States (Focazio et al. 2008). The sources of groundwater pollution can be the water runoff from sewer systems, the leakage from rivers, and the use of fertilizers and agrochemicals (Jurado et al. 2012; Vàzquez-Suñé et al. 2007). Another probable source of ECs in drinking water is the migration of chemical compounds such as phthalates, bisphenol A, or bisphenol A diglycidyl used in the epoxy resins or paints as inner coating materials in water tanks and tubbing pipes to the water (Casajuana and Lacorte 2003). The occurrence of ECs in drinking water is due to the less efficiencies obtained in their degradation using the conventional drinking water treatment plants (Kleywegt et al. 2011).

In addition, the presence of ECs in bottled water is attributed to the migration of these compounds to the packing material (Casajuana and Lacorte 2003), representing a risk to people due to its quality (Wolf et al. 2004). In consequence, the appropriate analysis of this kind of water is required to detect the ECs.

Currently, there are not standardized analytical methods for determining steroids in tap and drinking water. The availability of a standardized, reliable, robust, and fast analytical method ensures the detection of steroids in different aqueous matrices. The sample preparation is a significant source of errors and matrix spiking is often used to determine the effects on sample preparation and analysis, which is done by adding a known quantity of a component to blank, samples, and standard solution that is similar to analyte, such as deuterated analog (Maggioni et al. 2013) or an isotopically labeled compound (Du et al. 2014). Although those compounds can accuracy determinate matrix effect, the disadvantage is their cost. The determination of E_2 and EE_2 in water samples involves frequently solid phase extraction (SPE) using columns packed with octadecylsilyl (Gibson et al. 2007; Gorga et al. 2015) or recently polymeric phases (Gilart et al. 2014), which offer higher reproducibility that those reverse phases based on silica. Then, those steroids can be analyzed by either gas chromatography–mass spectrometry (GC–MS) or liquid chromatography with a diode array detector (LC-DAD), simple mass spectrometry (LC–MS), or

Table 1 Physical and chemical properties of E2 and EE2

Compound	Formula (CAS)	Formula weight (g/mol)	PKa	Solubility in water (mg/L)	Solubility in ethanol (mg/mL)	Structure
17β-estradiol	C18H24O2 (50-28-2)	272.38	10.4	1.51[a]	50[b]	
17α-ethinylestradiol	C20H24O2 (57-63-6)	296.40	10.5 to 10.7	9.20[a]	50[b]	

[a]Shareef et al. (2006a, b)

[b]Sigma-Aldrich (certificate of analysis)

lately with high-resolution quadrupole time-of-flight mass spectrometry (Q-TOF/MS). In comparison with LC techniques, gas chromatography analysis requires the chemical conversion of steroids from its low vapor pressure, which has been carried out with several substances, such as *N*-tert-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) with 1% tert-butyldimethylsilylchlorane (TBDMSC), *N*,*O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylsilylchlorane (TMSC), and *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide to improve thermal stability (Gibson et al. 2007) and to enhance the gas chromatography–mass spectrometry (GC–MS) analysis.

The limits of detection and quantification of the LC-DAD are in the order of mg mL^{-1} (Vallejo-Rodríguez et al. 2011), but it is necessary to achieve more sensitivity to detect traces of steroids in water from environmental matrices. LC–MS and LC–Q-TOF/MS have been used by various researchers (Gorga et al. 2015, Du et al. 2014, Chia-Yang et al. 2007). It is important to highlight that these techniques are robust, but at the same time costly, limiting the access to infrastructure. Hence, an available option to analyze ECs is the GC–MS equipment which balances cost and reliability.

Therefore, the aim of this work is the implementation of a reliable analytical method to determine E_2 and EE_2 in tap and bottled drinking water by optimization of analytical conditions to exhaustive extraction from sample water, efficient derivatization, and dependable identification and quantification by gas chromatography–mass spectrometry.

Methods

Study Context

The Metropolitan Zone of Guadalajara (MZG) has 4,796,603 inhabitants with an average population density of 1754 inhab/km^2 and is the third economical city in Mexico. The main source of drinking water of MZG comes from Lake Chapala with previous treatment and municipal drinking water plants provide 62% of water from this source (CEAS 2017). Lake Chapala, located in Jalisco, is the largest water body in the country and is a reservoir that receives discharges from Lerma River that collects the domestic and industrial wastewaters along its route of 708 km from Toluca Valley in the southwest of Mexico City, wastewater without treatment from the villages settled around Lake Chapala, runoff water from agricultural field, and discharges from wastewater treatment plants with low efficiencies. In consequence, quality and availability of water in Lake Chapala have been significantly affected (Brooks et al. 2003). The bottled water samples were obtained from the local markets of the MZG. The sources of the main bottled water brands of MZG are unknown but it can be assumed that those are wells.

Chemicals and Reagents

Two steroids, E_2 (purity > 98%) and EE_2 (purity > 98%), the surrogate standard 1,1-dichloro-2,2-bis(4-chlorophenyl)ethene (DDE, purity > 99%), and pyridine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Derivatization was performed using *N*,*O*-bis (trimethylsilyl) trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) from Supelco (Bellefonte, PA, USA). SPE cartridges packed with 0.5 g of octadecyl (C_{18}) were purchased from Phenomenex (Torrance, CA, USA). HPLC grade organic solvents (acetone, methylene chloride, methanol, and pyridine) were acquired from Fermont (Monterrey, Mex.). All connections and pipes used for the load of samples were made of polytetrafluoroethylene (PTFE). Ultrapure water was prepared by Milli-Q purifier system and N_2 chromatographic grade was obtained from a local supplier (INFRA, Guadalajara, Mex.).

Preparation of Stock, Working, and Calibration Solutions

Steroid solutions and surrogate compounds were prepared separately in methylene chloride at the concentrations of 100, 270, and 200 mg/L for E_2, EE_2, and DDE, respectively. The stock solutions were stored at −20 °C. The working solutions were obtained by suitably diluting the stock solutions with methylene chloride before use. DDE working solution was prepared at a concentration of 5 mg/L and the mixture of steroid solutions was prepared at a concentration of 2 mg/L. Seven calibration standards from 1 to 200 µg/L were obtained by suitable dilution and optimized derivatization of steroids. Then DDE was added to each calibration curve at a final concentration of 2500 µg/L. A DDE standard solution at a concentration of 2500 µg/L, without derivatization, was prepared afresh before each GC–MS analysis.

Sample Preparation

Tap water samples were obtained from municipal water supply system from Guadalajara, Jalisco, Mexico. Samples of bottled drinking water were selected among the most consumed brands in the local market. Water samples [ultrapure, tap (1 L) and drinking (1.5 L)] were filtered through a nylon filter (Millipore, 0.45 mm) and passed through C_{18} cartridges at a constant flow rate of 5 mL/min by applying vacuum. Then the cartridges were dried under vacuum for 20 min and eluted with methylene chloride. The solvent of the extract was reduced using an RV10

rotary evaporator (IKA) at controlled pressure and temperature. Extracted steroids from the water samples were dried with a gentle stream of nitrogen, derivatized with BSTFA + 1% TMCS, and kept at −20 °C for analysis.

GC–MS Analysis

Samples were analyzed using a model 6890 gas chromatograph (GC) from Agilent Technologies coupled with a model 5975 mass spectrometer (MS) and a quadruple mass filter with a model 7683 autosampler. The GC was equipped with an HP5MS 30 m × 0.25 mm capillary column (Agilent, USA), having 0.25 mm internal diameter with a stationary phase of 5% phenyl and 95% dimethyl polysiloxane, and 0.25 µm film thickness. The oven temperature program was as follows: 120 °C for 20 min, ramped at 15 °C/min to 250 °C, and finally increased at a rate of 5 °C/min up to 300 °C and held for 5 min. The injector temperature was 300 °C in splitless mode using an injection volume of 1.0 µL. Helium (99.999%, INFRA) was used as the carrier gas at a constant flow rate of 1.0 mL/min. Mass spectra were obtained by electron impact (EI) at 70 eV using an ionization source at 200 °C. Mass scanning was used in SCAN mode for optimizing the separation and identification of compounds. The most abundant *m/z* ratios were selected, which were 416 and 285 for E_2 (Di-TMS-E_2) and 425 and 285 for EE_2 (Di-TMS-EE_2), respectively. Selected ion monitoring (SIM) mode was utilized for quantification.

Optimization of Analytical Conditions

In this research, the proposed methodology involved the spiking of the analyte in the aqueous sample to evaluate the efficiency of extraction, concentration, and derivatization of steroids. Then the optimized method was used in the detection on tap and drinking water. For solid phase extraction optimization, DDE compound (2.5 µg) diluted in methylene chloride (12 mL) was put on the C_{18} cartridge and passed through it. Six aliquots of 2 mL was collected in vials and these were analyzed by GC–MS finding the minimum volume of methylene chloride to elute the steroids. Previously, C_{18} cartridges were conditioned with 6 mL of methanol and 6 mL of ultrapure water by gravity. To obtain optimal conditions of extract concentration, the extract solvent was reduced to approximately 0.5 mL using a rotary evaporator RV10 (IKA) at controlled pressure and temperature. Reduction efficiency was evaluated with methylene applying three different temperatures (30, 35, and 40 °C). Concentrated extracts were adjusted to 1 mL. An additional organic solvent to facilitate the dissolution of compounds with drying using a low steam pressure was required to reduce the losses of analyte during the reduction. The reduction and drying processes were evaluated using a standard solution (5 µg/L DDE) and three solvents (methanol, acetone, and methylene chloride). Experiments were performed in duplicate with each solvent. The dried extract was suspended in 100 µL of pyridine and 100 µL BSTFA + 1% TMCS, and then the mixture was homogenized with a vortex and placed in a Bransonic 5510 ultrasonic bath for 30 min at 60 °C (Shareef et al. 2006b). Preliminary experiments of steroid derivatization with and without pyridine were performed to assess the reaction formation efficiency of byproducts and also to evaluate the effect of the solvent.

Method Performance

In order to verify the performance of the optimized analytical conditions, quality parameters such as linearity, recovery, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision were evaluated (Miller and Miller 2010). These parameters were obtained by calibration curve analysis in ultrapure spiked water matrix, with adding 2500 µg/L of DDE as a surrogate prepared in duplicate with eight concentrations of steroids from 1 to 200 ng/L. The recovery was based on surrogate compound (DDE) and was previously determined by measuring the peak area responses from the DDE solution standard with those from spiked samples with the same concentration of DDE. Peak area ratios for each solution of steroids against its corresponding concentration were measured, and then a calibration curve was obtained from the least squares linear regression with its correlation coefficient. However, heteroscedastic behavior of variance throughout the experimental points of the calibration curve was demonstrated (95% confidence) by statistical F (Fisher) test , and weighted regression was used (Miller and Miller 2010). Accuracy and precision were evaluated with intra-spiked samples. Accuracy was determined by regression and concentration was measured as a percentage of the target (spiked) concentration. The coefficient of variation (CV) of the regressed concentrations was used to calculate the precision.

Steroids in Water

The analytical method was applied to determine two steroids in tap water and in six brands of drinking water by duplicate. Drinking water samples were randomly selected from local market and these were the most consumed by population. Samples were stored at 4 °C. Sampling of tap water was done taking one liter per hour for eight hours on one day in the supply tubing.

Results and Discussion

Optimization of Extraction Conditions and Reduction of the Extract

Efficiency of the Eluting and Volume Solvent

The solvent volume optimal to elute 99% of the steroid mass was 10 mL. DDE analyte was concentrated in the first fraction and decreased in the later fractions, so that the optimum volume for elution was chosen. The choice of solvent volume met the criteria recommended by Thurman and Mills (1998), when 90–100% of the analyte passes through the absorbent for the assayed solvent.

Assessment of Reducing Conditions on Rotary Evaporator

Reduction conditions of extract on a rotary evaporator were established by direct analysis (without derivatization) for the surrogate standard (DDE) in methylene chloride solution. DDE was concentrated to one-twentieth of its initial volume at three different temperatures in triplicate. An acceptable recovery percent and SD and CV minors were employed as the criteria of selection. Hence, the optimal temperature of reducing conditions was at 35 °C on rotary evarator and 100% of recovery and 6.6% of SD and CV between replicates was reached (data not shown).

Optimization of Derivatization Conditions

Evaluation of the Drying Process with Nitrogen

Methanol has a longer drying time (35 min) and recovery to 120% (SD = 8.6 and CV = 5.2), generating standard deviation and coefficient of variation larger than those of the other two solvents, and hence it was discarded. Acetone and methylene chloride produced the same drying time (27 min) and recovery of 101% (SD = 4.1, CV = 4.0) and 93% (SD = 3.2, CV = 3.4), respectively. Methylene chloride has less variation between repetitions so it was used as a solvent for steroid elution.

Solvent Effect

Without using any solvent, different compounds are formed by derivatization of EE_2 with BSTFA + 1% TMCS (Fig. 1a), which could reduce the efficiency of the reaction and skew the quantification (Zhang and Zuo 2005). Figure 1b, c, d show the mass spectra for byproducts corresponding to mono-TMS-E_1 (peak b), mono-TMS-EE_2 (peak c), and di-TMS-EE_2 (peak d). In general, mono-TMS-EE_2 is obtained by a reaction at the phenolic proton in the 3C position; di-TMS-EE_2 is formed by a reaction at both 3C and 17C positions of EE_2; finally, TMS-E_1 is formed by the breakdown product of mono-TMS-EE_2 (Shareef et al. 2006b). Mono-TMS-E_1 is formed in the absence of pyridine by derivatization of EE_2 (Fig. 1a) and its mass spectrum is shown in Fig. 1b (molecular ion at *m/z*, 342), with a retention time of 15.230 min. Furthermore, ion fragments of *m/z*, 285, 177, and 115 in the mass spectra of TMS-E_1 confirmed the silylation of OH functional group on the unsaturated ring in the steroid (Zuo and Zhang 2005). The ion at *m/z* 285 was attributed to the loss of $O{=}C_3H_5$ group from molecular ion *m/z* 342 $[M]^+$ on the last ring. The ion *m/z* 177 was attributed to $[(CH_3)_3{-}Si{-}O{-}C_6H_3{-}CH_2]^+$, the ion *m/z* 115 to $[(CH_3)_3{-}Si{-}O{-}C_2H_2]^+$, the ion *m/z* 73 to $(CH_3)_2{-}Si{=}O$), and the ion *m/z* 15 was due to the loss of a methyl group. Peak b of Fig. 1a is the mono-TMS-EE_2 with a retention time of 15.936 min, which was confirmed by its molecular ion at *m/z* 368. Fragment ions of mass spectra were at *m/z* 115 ($[(CH_3)_3{-}Si{-}O{-}C_2H_2]^+$), 177 ($[(CH_3)_3{-}Si{-}O{-}C_6H_3{-}CH_2]^+$), 232 ($[(CH_3)_3{-}Si{-}O{-}C_6H_3{-}C_4H_6]^+$), and 285 ($[M - 83]^+$), due to the loss of $HO{-}C_3H_5$ and ethynyl group from $[M]^+$ on the D ring. The mono-TMS-EE_2 was formed due to the reaction of hydroxyl group attached to the aromatic ring. Peak c of Fig. 1a is the di-TMS-EE_2 with a retention time of 16.728 min, which was confirmed by its molecular ion at *m/z* 440. Fragment ions of mass spectra were obtained at *m/z* 115 ($[(CH_3)_3{-}Si{-}O{-}C_2H_2]^+$), 196 ($[(CH_3)_3{-}Si{-}O{-}C_8H_9]^+$), 285 ($[M - 154]^+$) due to the loss of $SiO{-}C_3H_5$ and ethynyl group from $[M]^+$ on the D ring.

The BSTFA with 1% TMCS has been widely used as a silylation reagent. However, EE_2 has an ethynyl group at C-17, so it is necessarily a suitable catalytic solvent by derivatization with BSTFA + TMCS; this application allows the derivatizing reagent access to the hydroxyl group at C17 position (Shareef et al. 2006b). Pyridine is commonly used as a derivatization solvent provoking the activation of hydroxyl groups as a Lewis base (Zhang and Zuo 2005). Hence, pyridine was used in the derivatization of EE_2 with BSTFA + TMCS reagent and generating a silylated product (Zuo et al. 2007; Zuo and Lin 2007). In the present study, a ratio of 1:1 was used for BSTFA + TMCS/pyridine which is the same reported by Zhang and Zuo (2005). However, Shareef et al. (2006b) did not mention the used ratios for BSTFA + TMCS/pyridine. When pyridine was not used in the derivatization, Zhang and Zuo (2005) reported a formation of 43.3% for Mono-TMS E_1, 40.2% of Mono-TMS EE_2, and 16.4% of DI-TMS EE_2. The percentages of mono-TMS E_1, mono-TMS EE_2, and Di-TMS EE_2 that were formed in derivatization without pyridine were not measured but the presence of the three compounds was confirmed with the mass spectra. On the other hand, conversion of TMS-EE_2 to TMS-E_1 was

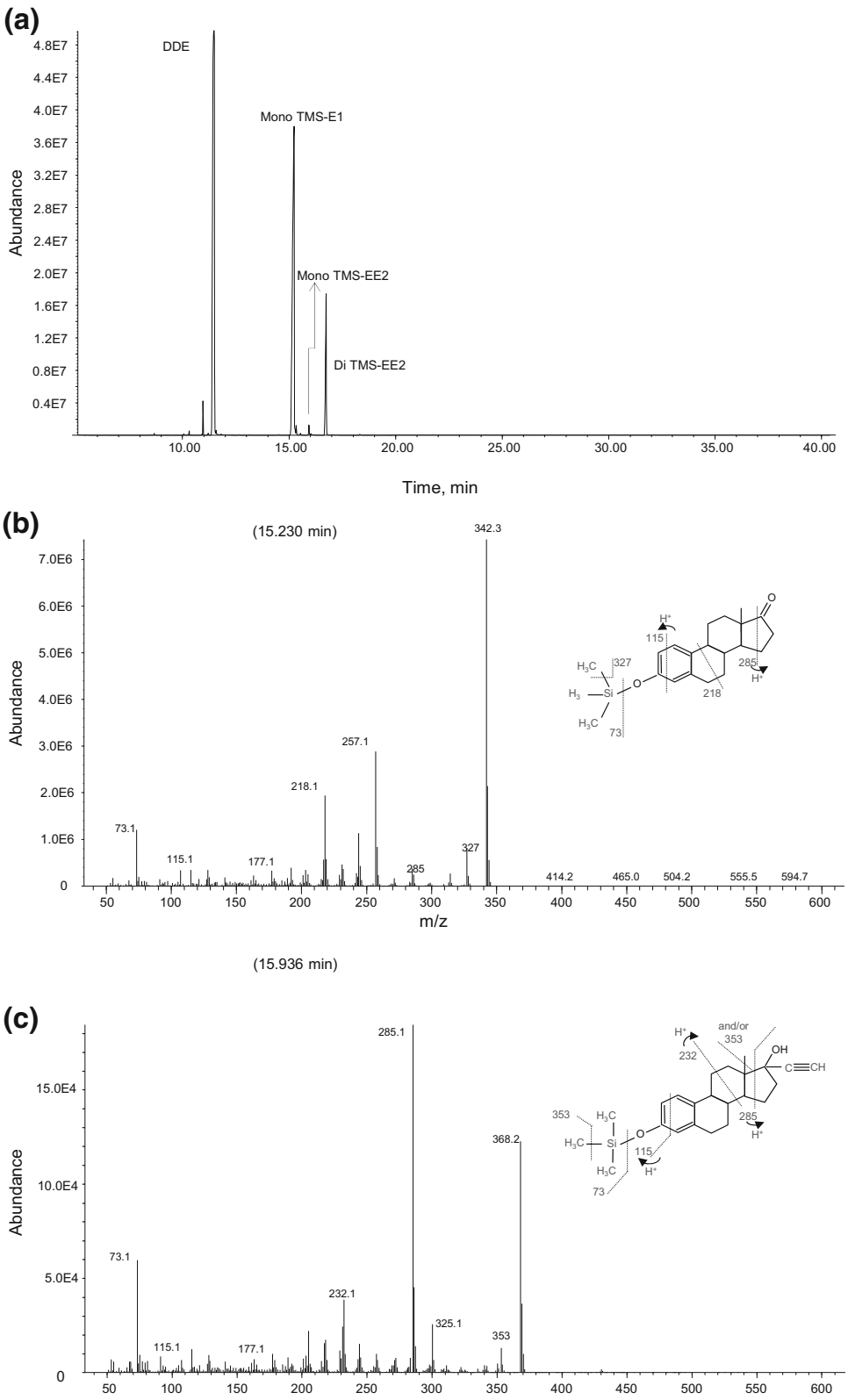


Fig. 1 **a** Chromatogram of the elution order of DDE and derivatized steroids by SCAN mode without pyridine: Mono-TMS E_1, Mono-TMS EE_2, and Di-TMS-EE_2 [(DDE) = 2500 μg L^{-1} and (EE_2) = 2500 μg L^{-1}]; **b** mass spectrum of Mono-TMS-E_1 Estrone; **c** mass spectrum of derivatized structure of Mono-TMS-EE_2 in **a**; and **d** mass spectrum of derivatized structure of Di-TMS-EE_2

prevented using pyridine (Shareef et al. 2006b), and the absence of derivatized estrone is confirmed in Fig. 2a. Therefore, the use of pyridine was essentially required in developing the method for both steroids, although there was not any evidence for the presence of this and other byproducts with E_2.

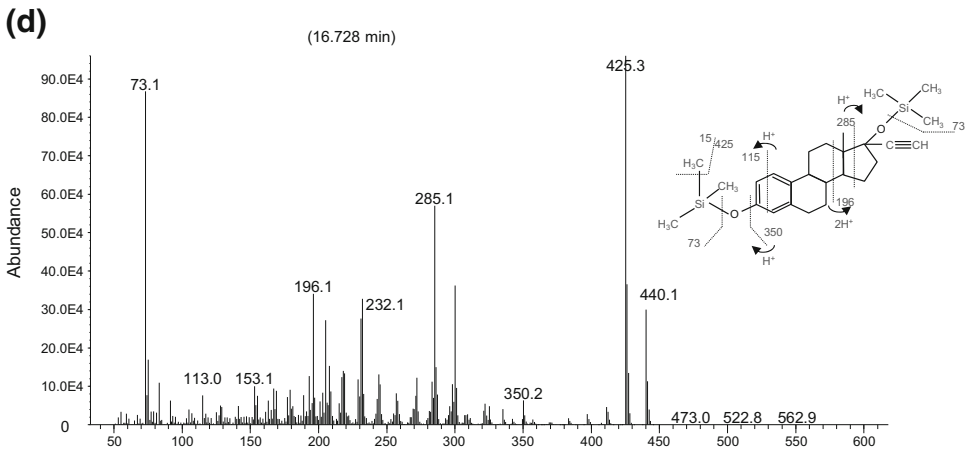


Fig. 1 continued

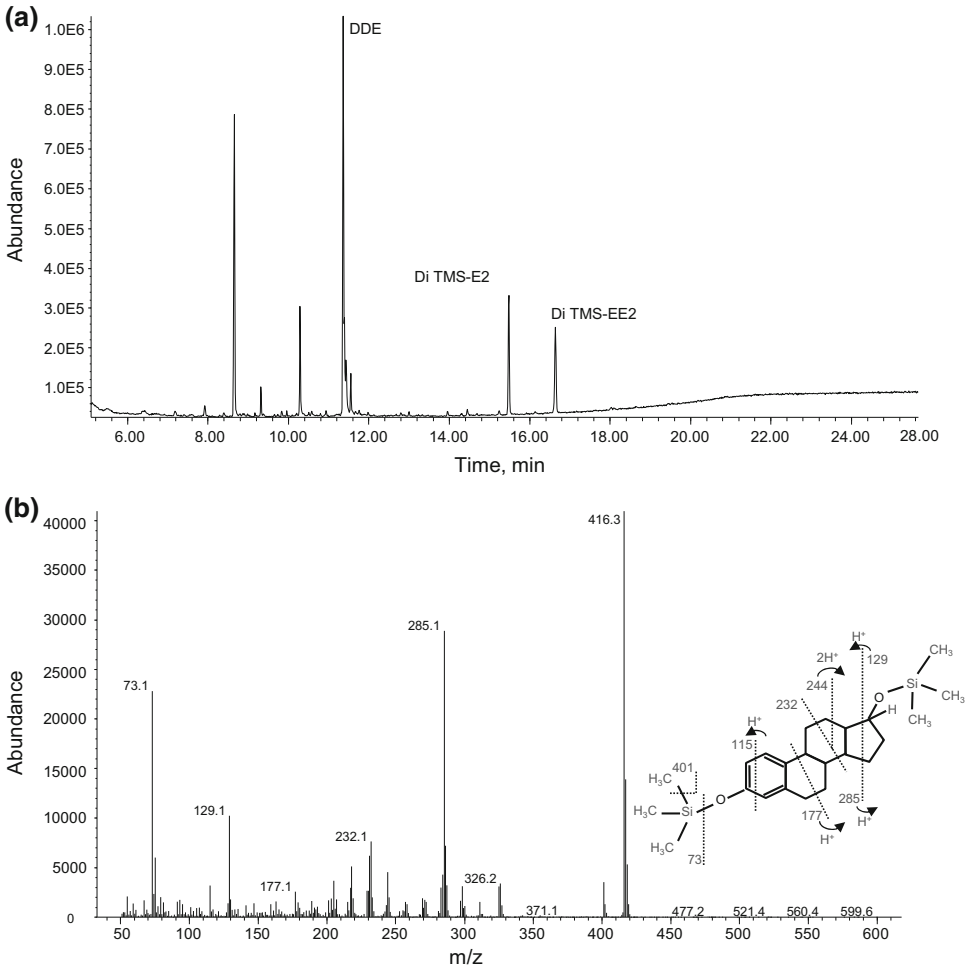


Fig. 2 **a** Chromatogram of the disappearance of Mono-TMS-E_1 using pyridine [(E_2, EE_2) = 200 µg L^{-1}, (DDE) = 2500 µg L^{-1}] and **b** mass spectrum of Di-TMS-E_2 (in pyridine)

Separation and Identification of Compounds by GC–MS

Figure 2a shows the order of separation of derivatized steroids with pyridine by GC analysis where the retention times for compounds were 11.528, 15.485, and 16.728 min for DDE, Di-TMS-E_2, and Di-TMS-EE_2, respectively. Figure 2b shows the mass spectra of Di-TMS-E_2. Chromatograms show the entire separation for derivatization. Fragment ions of mass spectra for the Di-TMS derivative of E_2 were at *m/z* 115 ($[(CH_3)_3–Si–O–C_2H_2]^+$), 129 ($[(CH_3)_3–Si–O–C_3H_5]^+$), 177 ($[(CH_3)_3–Si–O–C_6H_3–CH_2]^+$), 232 ($[(CH_3)_3–Si–O–C_6H_3–C_4H_6]^+$), and 285 ($[M - 83]^+$) due to the loss of HO–$C_3H_5$ and ethynyl

group from $[M]^+$ on the D ring and 401($[M - 15]^+$) due to the loss of methyl group. The separation between signals of derivatized compounds without co-elution demonstrates the selectivity of the chromatographic method (see Fig. 2a).

Assessment of the Analytical Method

Recovery

Efficiency of analytical method was based on surrogate standard recoveries. Optimized conditions were applied on seven water samples spiked approximately with 2500 µg/L of DDE. The results showed that the recoveries were 87% on average (SD ± 6.33, CV < 5.5%) for inter-spiked samples (Table 2). EPA method 1698 reported a recovery of 50 to 176% for E_2 and 50 to 126% for EE_2 using GC with high-resolution mass spectrometry (USEPA 2007). Therefore, the optimized method is reliable according to international standards due to the obtained analyte recoveries.

Accuracy of the Analytical Response

The accuracy of the analytical response was based on seven distinct concentrations of steroids per duplicate, which were measured after applying optimized procedures (extraction, concentration, and derivatization) on spiked water samples. The variation coefficient ranged from 3 to 18% for E_2 and from 4 to 16% for EE_2 (the value of level 8 was omitted) (Table 3). Recoveries ranged from 81 to 131% for E_2 (Table 3) on the order hand. However, the recoveries for EE_2 were lower than 70%. The probable degradation of EE_2 during derivatization may be the cause for its low determination in the analytical method. This phenomenon should be studied in future research.

Table 2 Recoveries and precision for spiked sample water with surrogate compound

Amount added (µg L^{-1})	SD	*R* (%)	CV (%)
2230	107.04	89	4.8
2150	118.25	86	5.5
2200	22.00	88	1.0
1980	25.74	79	1.3
2100	228.90	84	10.9
2200	72.60	88	3.3
2030	26.39	81	1.3

R (%) mean of recovery percentage based on surrogate standard (DDE), *SD* standard deviation, *CV* coefficient of variation

Linearity

The results showed for the analytical signal based on extracted, concentrated, and derivatized steroids fit significantly to a linear regression model in the range of experimental levels. Correlation coefficients (*r*) for E_2 and EE_2 were 0.96 and 0.91, respectively.

Limits of Detection and Quantification

The limits of detection and quantification were calculated from the weighted regression using the equations proposed by Miller and Miller (2010). Table 4 shows these values which are near to those previously reported in other studies. Liu et al. (2004) reported the LOD and LOQ values of 3.4 and 11.2 ng/L for E_2 and 0.8 and 2.6 ng/L for EE_2, respectively, in water samples. On the other hand, Zhang and Zuo (2005) reported a LOD of 2.50 and 2.20 ng/L for E_2 and EE_2 in superficial water, respectively. Recently, Maggioni et al. (2013) reported a LOQ of 3.13 and 3.00 ng/L for E_2 and EE_2 in drinking water, respectively. However, there are differences between the methods applied for the compared references. Conditions of analytical methods between our research and that of Liu et al. (2004) are the same, including the reagent BSTFA with 1% of TMCS but at different volumes; the cartridges used by Liu et al. (2004) were HLB Oasis for SPE. Zhang et al. (2011) used Oasis HLB cartridges for SPE and 30 µL MSTFA with 70 µL hexane for derivatization. Finally, Maggioni et al. (2013) utilized the quantification by electrospray ionization–liquid chromatography–tandem mass spectrometry without derivatization for steroids, a different method being used in the present study. Results were similar in spite of the kind of water and conditions of analytical method were different.

Estimation of Steroids in Water Samples

The analyses of drinking water samples revealed the presence of one or two of the selected steroids in each of the six brands, and both steroids were detected and quantified simultaneously on both of them. E_2 and EE_2 were found four times each in drinking water samples, that is, they were found in over 50% of the analyzed samples. Therefore, it is necessary to increase the number of analyzed bottled water samples and a more complete chemical analysis should be performed in the drinking water brands being sold in the region. No quantification of steroids in tap water samples (Table 5) was obtained in this study; it might be due to the presence of organic matter in water that had affected the retention of steroids on the phase of extraction cartridge and derivatization thereof. A good proportion of the compounds are found in conjugated form

Table 3 Accuracy and precision of analytical method

Spiked concentration (ng L^{-1})	Measured concentration ($n = 2$)					
	E_2			EE_2		
	Mean (ng L^{-1})	R (%)	CV (%)	Mean (ng L^{-1})	R (%)	CV (%)
200	161	81	10	107	53	4
100	80	80	18	54	54	11
75	63	84	5	40	54	16
50	48	95	5	32	65	13
25	20	79	12	14	56	9
10	13	131	10	6	65	4
5	6	116	3	–	–	–

R (%) mean of recovery percentage based on steroids, *CV (%)* coefficient of variation, *n* number of replicates, *E_2* 17β-estradiol, *EE_2* 17α-ethinylestradiol

Table 4 Limits of detection and quantification of analytical method (ng L^{-1})

Steroid	*n*	LOD	LOQ
E_2	7	1.0	3.0
EE_2	6	3.0	10.0

E_2 17β-estradiol, *EE_2* 17α-ethinylestradiol, *LOD* limit of detection, *LOQ* limit of quantification, *n* number of spiked samples

that was reported by Desbrow et al. (1998) and Suri et al. (2012) for the presence of inorganic salts, oxides, bases, and acids and for the reaction that occurs during the treatment of water. Furthermore, it should be noted that this is a representation of a small sample from a single sampling site. Treatment processes of water supply system are not as exhaustive and efficient as done with commercial water purification in emerging countries (Gleick and Cooley 2009). In spite of that, there were steroids in bottle water and their concentrations were similar to those reported previously by Velicu and Suri (2009) and Zhou et al. (2009) in surface water and Gibson et al. (2007) in spring water. The steroid concentration differences between the samples could be associated with the water supply source that is different for each brand of drinking water. Although water is purified for all bottles, some of them are not free of steroids.

This research has some limitations to be considered. One of the most important limitations of this study is the analytical instrument of GC–MS; it is difficult to reach the detection limit in the order of ng/mL for conjugated steroids in water such as estradiol-17-glucuronide, estrone-3-sulfate, and estradiol-17-acetate being one disadvantage for equipment which is possible using liquid chromatography/mass spectrometry (LC/MS) with atmospheric pressure chemical ionization (APCI) in mode positive ion (PI) or liquid chromatography/tandem mass spectrometry (LC/MS/MS) with selected reaction monitoring (SRM) (Díaz-Cruz et al. 2003). Another limitation of the research is the organic interferences of tap water that were not eliminated in solid phase extraction, which is recommended by Gibson et al. (2007); it is an extra cleanup step in the analytical method to increase the recovery of steroids in the analysis, and this step was not initially considered in the analytical method.

Limits of detection and quantification in the order of ng/L were similar to the ones reported in literature. The obtained LODs and LOQs allow to determine the concentrations of steroids in drinking water but not in the tap water. It is necessary to improve the preliminary treatment of sample tap water before solid phase extraction to eliminate the organic matter.

Presence of steroids in bottled drinking water samples is a concern, because in recent years the consumption of this

Table 5 Concentration of steroids in water samples

Sample	Compound	Frequency of detection	Mean ng L^{-1}	Minimum ng L^{-1}	Maximum ng L^{-1}	SD ng L^{-1}
Drinking water	E_2	6	4.2	<LOQ	11.4	3.5
	EE_2	4	16.5	<LOQ	24.4	6.0
Tap water	E_2	0	NA	NA	NA	NA
	EE_2	0	NA	NA	NA	NA

NA not applicable, *SD* standard deviation, *E_2* 17β-estradiol, *EE_2* 17α-ethinylestradiol

product has increased in the local population, generating potential exposure to these compounds in direct and prolonged forms. Although yet to be verified, this situation can become a potential risk factor for people's endocrine systems, since it is known that concentrations below 1 ng/L EE_2 are capable of producing changes to the reproductive and morphological levels in small living species (Bila and Dezotti 2007). There are not international parameters for limits of the presence of steroids in drinking water. However, EPA published for public review a draft list of contaminants that are currently not subject to any proposed or promulgated national primary drinking water regulations but may require regulation. This draft list is the fourth Contaminant Candidate List (CCL 4) and it is currently under review (USEPA 2015). The presence of steroids in drinking water for direct consumption should be avoided under the principle of prevention, because there are no specific references to the effects of these on human health (Bila and Dezotti 2007; USEPA 2015). Therefore, the development and application of specific rules governing these compounds in water is very important.

Conclusions

A reliable, robust, and fast analytical method was developed for determining E_2 and EE_2 in tap water and commercial drinking water samples. Elution volume in solid phase extraction, reducing conditions on rotary evaporator, and derivatization conditions were optimized. The evaluation of the total analytical method conditions yielded high recovery efficiencies, with an average of 87%, which are in agreement with those reported by the EPA. Linearity of analytical method was greater than 90% in the range of experimental levels indicating a positive correlation between the amounts of the spiked analyte and its recovery levels. Finally, more studies about the determination of these steroids are required to confirm the present results and to propose a more efficient degrading method.

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