Steroid hormones, inorganic ions and botrydial in drinking water : Determination with capillary electrophoresis and liquid chromatography-orbitrap high resolution mass spectrometry

El Fellah, Samira

2017-07

El Fellah , S , Duporte , G & Siren , H 2017 , ' Steroid hormones, inorganic ions and botrydial in drinking water : Determination with capillary electrophoresis and liquid chromatography-orbitrap high resolution mass spectrometry ' , Microchemical Journal , vol. 133 , pp. 126-136 . https://doi.org/10.1016/j.microc.2017.03.016

http://hdl.handle.net/10138/311081
https://doi.org/10.1016/j.microc.2017.03.016

Elsevier Editorial System(tm) for Water Research

Manuscript Draft

Manuscript Number:

Title: Progesterone and botrydial in drinking water: Analysis with capillary electrophoresis and identification with liquid chromatography-orbitrap high resolution mass spectrometry

Article Type: Research Paper

Keywords: progesterone; botrydial; capillary electrophoresis; high resolution liquid chromatography; orbitrap-mass spectrometer

Corresponding Author: Professor. Heli Marja Marita Siren, Ph.D.

Corresponding Author's Institution: University of Helsinki

First Author: Samira El Fellah, BSc

Order of Authors: Samira El Fellah, BSc; Geoffroy Duporté, Post-Doc; Heli Marja Marita Siren, Ph.D.

Abstract: Steroids, botrydial, inorganic cations, and inorganic anions were studied from cold and hot tap water samples with capillary electrophoresis (CE) using UV detection. Identification of the steroids and botrydial was made with ultra-high-performance liquid chromatography (UHPLC) coupled to electrospray ionization orbitrap high resolution mass spectrometer. Solid phase extraction with nonpolar and ion-exchange sorbents were used to enrich all the analytes except inorganic ions from two litres into 250 microliters to fulfil the method calibration limits in CE and UHPLC. The steroids identified from the drinking water samples were testosterone, androstenedione, and progesterone. Concentrations of progesterone in both cold and hot tap water samples from Helsinki households were from 0.031 ng/L to 0.135 ng/L and from 0.054 ng/L to 0.191 ng/L, respectively. Chloride and nitrate amounts were at 25 mg/L. The highest amounts of calcium, potassium, magnesium, and sodium were 20, 1, 1, and 17 mg/L, respectively. Copper, iron, sulphate, and ammonium were below the method limit for the concentration ranges. The biocultivation of the mold was identified by finding botrydial from all drinking water samples. Its amount in both cold and hot tap water samples was 1900% higher than in the reference tap water (originating from well).

Suggested Reviewers: Merike Vaher PhD
Analytical Chemistry, Tallinn University of Technology
merike.vaher@ttu.ee
Senior research chemisist in analytical chemistry
Expetice on water chemistry; separation technoogy

Dzintars Zacs
Chemistry, University of Latvia
dzintars.zachs@gmail.com
Expert in water research.

Xiaoping Wu
Fuzhou University

wapple@fzu.edu.cn
water research using capillary electrophoresis

Joselito P Quirino
University of Tasmania
jquirino@utas.edu.au
Liquid Chromatography and Capillary Electrophoresis

Cover Letter

To

The Editor of

Water Research

Helsinki 9.11.2016

Dear Editor,

Please find our manuscript on

Progesterone and botrydial in drinking water: Analysis with capillary electrophoresis and identification with liquid chromatography-orbitrap high resolution mass spectrometry

written by Samira El Fellah, Geoffroy Duporté, Heli Sirén

With the submission of this manuscript, I would like to undertake that the above-mentioned manuscript has not been published elsewhere, or sent for publication to other journals.

The manuscript has three (3) tables and five (5) figures.

The authors declare that they do not have competing financial interest concerning the project. They do not have any conflicts, either. The corresponding author of the paper is Heli Sirén, University of Helsinki, Department of Chemistry, Finland.

The main idea of the research project was to detect male and female hormones in tap water. Because of the low concentrations in purified water, the water samples were concentrated with tandem-SPE methodology. Capillary electrophoresis was used for quantification of male and female endogenic hormones. Ultra-high-performance liquid chromatography (UHPLC) coupled to electrospray ionization orbitrap high resolution mass spectrometer was needed to identify the analytes.
In addition, we found precursors of mould in all samples. Botrydial was identified with the MS and MS^2 systems to have the accurate masses for the analytes.

According to literature, there are studies on quantification of steroids with LC and GC, but not with the similar CE technique and the new sample handling process as we have described.

In addition, the metabolites are as in the waters of the plants (no hydrolysis). Then, we made a new concept for water treatment to catch the steroids and their glucuronide conjugates. When using the new polymer material, we recognised that the supernatant needed treatment to find out whether the steroids were completely adsorbed.

The authors declare that they do not have competing financial interest concerning the project. They do not have any conflicts, either.

Thank you for considering the submission. I look forward to your response.
Sincerely

Heli Sirén
Adjunct Professor in Analytical Chemistry
University of Helsinki, Finland

cc. The Abstract sheet of the paper.

Progesterone and botrydial in drinking water: Analysis with capillary electrophoresis and identification with liquid chromatography-orbitrap high resolution mass spectrometry

Samira El Fellah, Geoffroy Duporté, Heli Sirén*)

University of Helsinki, Department of Chemistry, Laboratory of Analytical Chemistry, PO Box 55 (A.I. Virtasen aukio 1), FI-00014 University of Helsinki, Finland
*Correspondence: Heli Sirén, University of Helsinki, Department of Chemistry, A.I. Virtasen aukio 1, PO Box 55, FI-00014 University of Helsinki, Finland (heli.m.siren@helsinki.fi)

Highlights

1. Partial-filling micellar electrokinetic chromatography method used for the determination of steroids and a marker compound of mould in cold and hot tap water samples.

2. UHPLC-MS^2 was used for identifying the analytes in extract made with tandem-SPE system.

3. A new repeatable analysis system was developed for the detection of nutrients and human steroids.

4. The study has a novelty value, since simultaneous research has not been earlier reported with capillary electrophoresis and ultra-high performance liquid chromatography.

Abstract

Steroids, botrydial, inorganic cations, and inorganic anions were studied from cold and hot tap water samples with capillary electrophoresis (CE) using UV detection. Identification of the steroids and botrydial was made with ultra-high-performance liquid chromatography (UHPLC) coupled to electrospray ionization orbitrap high resolution mass spectrometer. Solid phase extraction with non-polar and ion-exchange sorbents were used to enrich all the analytes except inorganic ions from two litres into 250 microliters to fulfil the method calibration limits in CE and UHPLC. The steroids identified from the drinking water samples were testosterone, androstenedione, and progesterone. Concentrations of progesterone in both cold and hot tap water samples from Helsinki households were from 0.031 ng/L to 0.135 ng/L and from 0.054 ng/L to 0.191 ng/L, respectively. Chloride and nitrate amounts were at 25 mg/L. The highest amounts of calcium, potassium, magnesium, and sodium were 20, 1, 1, and 17 mg/L, respectively. Copper, iron, sulphate, and ammonium were below the method limit for the concentration ranges. The biocultivation of the mold was identified by finding botrydial from all drinking water samples. Its amount in both cold and hot tap water samples was 1900% higher than in the reference tap water (originating from well).

Keywords: progesterone, botrydial, capillary electrophoresis, liquid chromatography, orbitrap.

Manuscript_November 10th, 2016

Water Research

Progesterone and botrydial in drinking water: Analysis with capillary electrophoresis and identification with liquid chromatography-orbitrap high resolution mass spectrometry

by Samira El Fellah, Geoffroy Duporté, Heli Sirén

Highlights

- Novelty value: Profiling of cold and hot tap water samples.
- CE-UV was used for determination of steroids and metabolites of *Botrytis cinerea.*
- UHPLC-MS^2 was used for identifying extracts made with a tandem-SPE system.
- Progesterone was found at 0.031 ng/L - 0.191 ng/L in tap waters of Helsinki.
- Botrydial was 1500% higher in communal water than in tap water from a well.

Progesterone and botrydial in drinking water: Analysis with capillary electrophoresis and identification with liquid chromatography-orbitrap high resolution mass spectrometry

Samira El Fellah, Geoffroy Duporté, Heli Sirén*)

University of Helsinki, Department of Chemistry, Laboratory of Analytical Chemistry, PO Box 55 (A.I. Virtasen aukio 1), FI-00014 University of Helsinki, Finland

*Correspondence: Heli Sirén, University of Helsinki, Department of Chemistry, A.I. Virtasen aukio 1, PO Box 55, FI-00014 University of Helsinki, Finland (heli.m.siren@helsinki.fi)

Abstract

Steroids, botrydial, inorganic cations, and inorganic anions were studied from cold and hot tap water samples with capillary electrophoresis (CE) using UV detection. Identification of the steroids and botrydial was made with ultra-high-performance liquid chromatography (UHPLC) coupled to electrospray ionization orbitrap high resolution mass spectrometer. Solid phase extraction with nonpolar and ion-exchange sorbents were used to enrich all the analytes except inorganic ions from two litres into 250 microliters to fulfil the method calibration limits in CE and UHPLC. The steroids identified from the drinking water samples were testosterone, androstenedione, and progesterone. Concentrations of progesterone in both cold and hot tap water samples from Helsinki households were from 0.031 ng/L to 0.135 ng/L and from 0.054 ng/L to 0.191 ng/L, respectively. Chloride and nitrate amounts were at 25 mg/L. The highest amounts of calcium, potassium, magnesium, and sodium were 20, 1, 1, and 17 mg/L, respectively. Copper, iron, sulphate, and ammonium were below the method limit for the concentration ranges. The biocultivation of the mold was identified by finding botrydial from all drinking water samples. Its amount in both cold and hot tap water samples was 1900% higher than in the reference tap water (originating from well).

Keywords: progesterone, botrydial, capillary electrophoresis, liquid chromatography, orbitrap.

1 Introduction

Water purification plants process drinking water. They have all responsibility for the clean water in circumstances, when the water enters to the fitting pieces of the connecting pipes that are coupled on-line with those of the premises (Ministry of Social Affairs and Health, 2000). The absolute control of household waters belongs to water users and residences.

The authorities control and audit the water production at water purification plants (Kaunisto, 2002; WHO, Guidelines for drinking-water quality, 2011). Water quality may change in the pipelines leading from the plant to the users. In the pipelines, if the water consumption and the hydrostatic pressure are small, the water flow is weak. This may agitate formation of precipitation that produces adsorption of nutrients and decant nutritious material for substrate of microbes and mould. Drinking water must not cause corrosion or form harmful precipitation in pipelines or other water devices because of the possible effects on human health. Mainly, the quality demands and recommendations of water are applied to health effects lacking the technical quality of water (Mons et al. 2013). The criteria for individual compounds, which are extracted from the polymers and which dissolve in drinking water, is important. According to European Community (EU), the largest population depending on their own wells and that is totally out from the water services, is in Finland (Hiisvirta, 2001). The raw water differs from that of general in EU (WHO, Guidelines for drinking-water quality, 2011) because it contains humus. Anyhow, regardless of it, the water is used as drinking water without water treatment if it fulfils the quality for health issues.

In the 2100 century Finland, copper-made water pipes were changed into polymeric crosslinked polyethylene (PEX, crosslinked high-density polyethylene) water piping systems (Parliament of Finland, 2000; WHO, Guidelines for drinking-water quality, 2011). The cold water pipes are made of cast iron, galvanized, stainless, and acid resisting steel, and from copper (Mäkinen, 2008). Currently, hot water pipes are made of copper or stainless and acid resisting steel. The polymer

materials, polyvinyl chloride (PVC), polyethylene materials (low, high, and medium density, PEL, PEH, and PEM), polybutylene (PB), and crosslinked high density polyethylene (HDPE) polymer (PEX) are suitable for pipes of cold water, but PB and PEX polymers are used for pipelines of hot water use (WHO, Guidelines for drinking-water quality, 2011).

When in contact with drinking water, polymers are classified as raw materials, additives, modifiers, or products that need to fulfil the demands of Food Regulations (Food Safety Regulations, 1989). Even so, most of the fixed structures and devices in drinking water plants and network systems are out of the coverage with this regulation. In respect of health issues, the requirements for the individual compounds in polymer dissolving in drinking water are important. Decrease of corrosion and microbe activity in water are not highly prioritized although they may have a significant role in quality of food processes, where water is added as an external solvent to the production (WHO, Guidelines for drinking-water quality, 2011).

Existence of steroid hormones in environmental water is in interest because hormones are produced naturally in human body or they are chemically synthetized and after in-take, excreted with body fluids. They are slightly water-soluble making their medicinal activity long-lasting (Arditsoglou and Voutsa, 2010; Sirén and El Fellah, 2016a).

Worldwide, trace amounts of birth control medications are present in many urban and suburban water supplies. The occurrence of endocrine disrupting compounds (EDCs) in drinking water sources has raised public attention. Determination of endocrine-disrupting compounds in drinking waters have mainly been studied by fast liquid chromatography–tandem mass spectrometry and gas chromatography-mass spectrometry (Magi et al., 2010; Barreiros et al., 2016). According to literate, chlorination does not break down these endogenous steroid hormones (Lubick, 2008). The presence of the hormones 17β-estradiol, 17α-ethinylestradiol, estriol, estrone, and progesterone has been reported in surface waters and ground waters in the United States of America (Velicu and Suri,

2009), Europe (Aydin and Talinli, 2013), Asia (Chang et al., 2008), and Australia (Scott et al, 2014) at concentrations up to 180 ng/ L and even more (Sirén and El Fellah, 2016a). The increasing evidence of their impact (Thuy and Nguyen, 2013) has led to the recent inclusion of 17β-estradiol and 17α-ethinylestradiol in the first tracking list of harmful ECDs in EU (EU directive, 2013). 17β-estradiol, 17α-ethinylestradiol, estrone, and estriol are also included in the contaminant candidate list of the United States Environmental Protection Agency. The list comprises compounds that are suspected to require regulation under the Safe Drinking Water Act (EPA, Drinking Water Regulations and Contaminants, 2009). The likelihood for regulations and their detection in treated waters may probably be strengthening in the future (Kuster et al., 2008). Therefore, the demand to develop new treatment technologies which allow utilities to produce treated water for drinking with higher quality, has increased.

However, the big issues are the steroid drifts of which exact sources to water intakes of purification plants are not known. In fact, the legislation concerning quality of water is wide. Water purification is not standardized and regulations do not exist on how to audit and check the water pre-treatment procedures and fairly inform the households. The source of intake water effects significantly on system set-up of the pre-treatment and cleaning procedures but also on the analysis techniques, since the effectivity of water purification changes from process to process (Silva et al., 2012). Anyhow, steroids are supposed to be removed from wastewater during biological secondary treatment, although there is evidence on their existence after that procedure. Thus, the sludge removes steroids from water. In spite of that, estrogens have the most efficient degradation process in active carbon plants, where the lifetime of sludge is over 10 days (Radjenović et al., 2008). Steroids can be identified with separation techniques only from the concentrate of drinking water because their quantities are at ng-µg/L level.

The increase of temperature and pH of biosolids after lime addition may cause transformations in steroid hormones. For instance, conjugated hormones may be cleaved, which would increase free

hormone concentrations in limed biosolids. In addition, since progesterone is a precursor to hormones in organismal biosynthesis, transformations of other steroid hormones into progesterone could increase progesterone levels (Bevacqua et al., 2011).

Progesterone was chosen for one of the monitored compounds in our study, since it was detected at high amounts in influent and effluent water samples of wastewater purification plants (Sirén et al., 2016). The pK_a of progesterone is 18.92/−4.80 (pKa value of strongest acidic/strongest basic (ChemAxon, 2011) and water solubility 8.8 mg/L (DrugBank, 2016). However, it was found that progesterone has a log K_{ow} of 3.87 (Neale et al., 2009).

Since progesterone has high octanol-water partitioning coefficient, a greater portion of it should be sorbed onto sludge and soluble particles than be present in aqueous phase. Lately, it was also observed that progesterone levels in coarse lime amended biosolids are significantly higher ($P < 0.05$) than those amended with fine (passing through 2 mm sieve) lime (Bevacqua et al., 2011).

Earlier, chemical monitoring of the biocultivation of mould has not been published when capillary electrophoresis has been used as the method. Botrydial, 3-acetylbotcinic acid, cotcinin A, and abscisic acid are metabolites isolated from *Botrytis cinerea*, and therefore those marker compounds will identify the mould (Pinedo et al., 2016). The effect of liming on antibacterial and hormone levels in wastewater biosolids (Oleszewski et al., 2013). The study results suggest that coarse lime might be more active than fine lime due to less interaction with surrounding air.

The present work was done to study drinking water used in households in Helsinki and in the neighbouring areas. The idea was to study steroid hormones in cold and hot tap water. The inorganic anions and metal cations were determined, since metals are all very dependent on the chemical forms in which they occur in the nature. The analyses were done with three capillary electrophoresis methods, one of which was a partial filling micellar technique (PF-MEKC) for on-line concentration of the analytes to detect them in direct UV-mode and. The two other methods were zone electrophoresis methods with indirect-UV detection modes for metal ions and inorganic

anions. The mould indicator, botrydial was detected with the PF-MEKC method. The observed analytes were identified with ultra-high-performance liquid chromatography coupled to an orbitrap high resolution mass spectrometer (UHPLC-HRMS) to obtain accurate masses for the unknown analytes.

2 Experimental

2.1 Chemicals

Progesterone ($C_{21}H_{30}O_2$, assay ≥ 98%), testosterone ($C_{19}H_{28}O_2$, assay ≥ 98%), 1,3,5(10)-estratrien-3,17-β-diol 3-glucosiduronate ($C_{24}H_{32}O_8$, TLC grade), and androstenedione ($C_{19}H_{26}O_2$, assay ≥ 98%) were purchased from Sigma-Aldrich (Germany). The steroids were used as received, and stored in a dark and cold room (+ 4 ^{o}C). Botrydial standard was not available. Therefore, it was identified with the exact mass method with UHPLC-HRMS.

Other chemicals were ammonia (min. purity 25%) from VWR International S.A.S (France), and ammonium acetate (98%, AA) from Sigma-Aldrich (Germany), and diethyl ether (GC assay, min 99.5%) from Merck (Germany). Methanol (HPLC grade) was from Fisher Scientific (UK) and ethyl acetate (GC assay > 99.5 percentage) from Sigma-Aldrich (Germany). The sodium salt of taurocholic acid monohydrate (BioXtra, ≥ 95% (TLC)) and sodium dodecyl sulphate (approx. 99%) were from Sigma-Aldrich (Germany). Hydrochloric acid (1.0 M, analysis result 0.9995 mol/L, ±0.0021 mol/L) and sodium hydroxide (1M, analysis result 1.0003 mol/L, ± 0.0021 mol/L) were purchased from Oy FF-Chemicals Ab (Finland). Methanol was also used as the solvent in standards and as the marker of electroosmosis. Pyridine (>99.8%), glycolic acid (>99%), and 18-crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane, > 99%) were from Sigma-Aldrich Finland Oy (Helsinki, Finland). The buffer solution pH 7.7 for HPCE separation of anions was from Fluka Chemie AG (Buchs, Switzerland). All waters used were purified with a Direct-Q UV Millipore water purification system (Millipore S.A., Molsheim, France).

2.2 Instruments and methods

2.2.1 Capillary electrophoresis and methods

A Hewlett-Packard 3D CE instrument (Agilent, Waldbronn, Germany) equipped with a photodiode array detector (190-600 nm) was used for the determination. The CE instrument was applied with ChemStation programmes (Agilent) for instrument running and data handling. Bare fused silica capillaries (i.d. 50 µm, o.d. 375 µm) were purchased from Polymicro Technologies (Phoenix, AZ, USA). They were cut to the total lengths (L_{tot}) of 80 cm and 60 cm with the efficient length (L_{eff}) of 71.5 cm and 51.5 cm for steroid and ion analyses, respectively. Before use, they were conditioned by sequentially flushing with 0.1 M NaOH, milli-Q water, and the electrolyte solution, for 20 min each at 13.634 p.s.i. (940 mbar). Before each analysis, the capillary was flushed with 0.1 M NaOH and the electrolyte solution 2-5 min depending on the method.

Three types of analysis methods were used: a partial filling micellar electrokinetic chromatography (PF-MEKC) and two capillary zone electrophoresis methods with indirect UV detection (CZE indirect-UV). The temperature during the analyses was +25 °C. Positive polarities and voltages of 25 kV and 20 kV were set as the constant value for steroid and inorganic cation analyses, respectively. Negative polarity with the voltage of 20 kV was needed for inorganic anion analyses. The electrolyte solutions in PF-MEKC were prepared to give 17 µA current. In the cation and anion analyses the current was between 30-40 µA. The analysis times were 20 min, 10 min, and 10 min for steroids, cations, and anions, respectively. To quantify steroids, cations and anions, the samples were injected with 0.50 p.s.i (34.5 mbar) for 75 s, and with 0.73 p.s.i. (50 mbar) pressure for 10 s and 5 s, respectively. In PF-MEKC, the steroids were detected at 214, 220, and 247 nm. Cations and anions were studied with indirect-UV detection at 200 nm, 214 nm, 220 nm, and 254 nm with the reference wavelength of 420 nm.

For a reference, inorganic cations in the water samples were determined using an ICP-AES (Iris Intrepid II XDL, Thermo Electron Corporation, UK). The flow gas was argon (99.998%). The data collecting and handling was made with Teva kp software. The gas used was technical air containing

N_2 and 20.9% O_2 and it was made of natural compressed air, acetylene (purity 99.5%) and nitrous oxide gas (purity 99.0%). The temperature of the flame was 3000°C and the flow of C_2H_2 gas was 4.3 L/min. All gases were from Aga Oyj (Espoo, Finland). Determination of metals in Imatra (TW-I), drilled well water in Lappeenranta (DW-L), drilled well water in Lake Saimaa area (DW-LS), tap water in Lappeenranta (TW-L), and a cold tap water from a household in Vantaa (TW-V) were measured with ICP-AES. The elements used for the measurement were the specific wavelengths of Cu 324.754{103} nm, K 766.491 nm, Mg 279.553{120} nm, and Na 589.592{57} nm (Sirén et al., 2015).

In determination of inorganic anions (SO_4^{2-}, Cl^-, NO_3^-), the ion chromatograph of Dionex DX-120 (Sunnyvale, California, USA) was used. The sample injections were made with 25 mm syringes with 45 µm polypropylene membranes. The column used was IonPac AS22 Analytical (4 mm × 250 mm). The guard column was Dionex OnGuard® II 1cc column, which removes Be, Mg, Ca, Sr, Ba, Ra, Mn, Fe, Ni, Cu, Zn, and Al from the samples. Detection was made with a suppressed conductivity detector CD 25. The suppressor was self-cleaning ASRS® ULTRA II 4 mm Autosuppression® in recycle mode with current of 31 mA. The injection volume was 10 µL. Flow rate of the eluent was 1.07 mL/min. Temperature during the measurements was kept at 20°C.

2.2.2 Electrolytes in capillary electrophoresis separation

The electrolyte solution in the partially filled micelle composition was 20 mM ammonium acetate (Sirén et al., 2008). Its pH was adjusted with 25% ammonia to pH 9.68. The final micelle mixture was prepared by adding 1000 µL of 20 mM ammonium acetate (AA) solution, 440 µL of 100 mM sodium dodecyl sulphate (SDS) made in 20 mM AA, and 50 µL of 100 mM sodium taurocholate solution together, in this specific order. The micelle and the electrolyte solutions were sequentially introduced into the capillary. The micelle plug was placed between the BGE solution and the standard or the sample solution. The technique was modified from Ref. (Sirén et al., 2008).

The electrolytes (BGE) and the separation method for cations are presented in our previous studies (Sirén et al., 2015; Rovio et al., 2011). The inorganic cations were analysed in electrolyte solution containing 9 mM pyridine, 12 mM glycolic acid, and 5 mM 18-crown-6 ether in milli-Q water (pH 3.6, adjusted with 0.1 M HCl). The electrolyte solution for inorganic anions was a commercial product. The pH values of the electrolyte solutions were checked using InoLab pH7110 (WTW) instrument. The electrodes were calibrated with commercial buffer solutions at pH 4.00, 7.00 and 10.00 (Fisher Scientific, Loughborough, UK).

2.2.3 Identification of the compounds by UHPLC coupled with a mass spectrometer

The compounds in the extracts were analysed with a Thermo Ultimate 3000 UHPLC coupled with an Orbitrap Fusion TMS (Tribrid mass spectrometer). An Acquility UHPLC BEH C18 column (50 x 2.1 mm, 1.7 µm, Waters, Ireland) was used for chromatographic separation. The eluents were A) 0.1% formic acid in milli-Q water and B) 0.1% formic acid in acetonitrile. The eluent flow rate was 0.6 mL/min. Gradient elution was used from 95:5 (v/v, A/B) by increasing B to 100% in 15 min and thereafter returning to the initial composition within one minute, and lastly keeping the composition for 4 min to equilibrate the system. Electrospray ionization was used in both positive and negative modes. The parameters for the mass spectrometer are listed in Ref. (Duporté et al., 2016). The orbitrap resolution used in this work was 120000. The MS analyses were made with both SCAN(+) mode at m/z 86.00 – 470.00 Da and EICs of the specific ion fragments.

2.3 Samples

2.3.1 Sampling

The drinking water samples were sampled on 7th -13th December 2015. Within the framework of the current study, six randomly chosen tap water samples were analysed for the content of selected steroids, inorganic ions, and marker for biocultivation of mould. Portions of cold tap water (1 x 2 L) and hot water (1 x 2 L) samples were taken from three households in Helsinki City centre (Etu-Töölö, Kumpula, Munkkiniemi) and two from the surrounding areas (Myyrmäki-Vantaa, Seutula-

Vantaa, Porvoo) of 50 km radius from Helsinki in Southern Finland. As references for the inorganic ions, the tap water samples were also taken from South-Eastern Finland (Lappeenranta and Imatra), because those analytes depend on the environment. One of the main samples was from Seutula from the well, all others were from pipelines build or constructed within 15 years. It should be noted that the pipelines of the University of Helsinki (Kumpula) were reconstructed in 2015. The sample origin covers the most significant drinking water in Finland, since the area has 1.2 million inhabitants.

Sampling was done into clean plastic bottles (2 L). The bottles for cold-water samples were prewashed with ultra-pure water and tap water was let to flow (3 x 2 L volume) before final sampling. They were completely filled with overflowing the water. For hot water sampling, the water was let to flow at least 10 mins at a maximum flow to stabilize the temperature in the sample. The sampling procedure was same as for cold water. When the samples were in the bottles, they tightly capped. The bottles were transported directly to the laboratory, while keeping them in cold (+4 °C) and dark place. The extraction procedure was performed not later than 3 days after the sampling.

2.3.2 Sample preparation with solid phase extraction

The SPE device Vac Master (Biotage® VacMaster^{TM} 20 Sample Processing Station) was used for solid phase extraction and concentration of the water samples. They were concentrated with Strata-X 33u polymeric reverse phase columns (non-ionic steroids: reverse phase, 500 mg / 6 mL, U.S.A.) and with amino (NH_2) polar phase columns (ionic steroids: 3 mL, amino (NH_2), aminopropyl silane, 40 µm APD, 60 Å) from J.T. Baker Inc. (The Netherlands).

In the laboratory the 2-L volume from the bottled water were used for analysis. The cold and hot tap water samples were pre-treated and analysed separately. First, they were filtrated with both glass fibre and membrane filters. Then, first the steroids were isolated with C_{18} nonpolar material (STRATA-X, 1 column/1 L water sample, 2 columns in total) and then the compounds left in the

eluate were extracted with amino material (1 column/ 200 mL water sample, 10 columns in total). The aim was to collect the non-adsorbed steroids and especially the anionic steroid glucuronides. Before use, the sorbents were washed with methanol and water. The amino sorbent was also washed with 0.1 M HCl before water. After sorbent conditioning and introducing the sample, the SPE materials were dried for 30 min under vacuum. The extraction was made with methanol in volumes of 6 mL and 3 mL for C_{18} and amino columns, respectively. The C_{18} column eluates obtained from the same sample were combined. Similarly, the procedure was done with the eluates from the amino columns. The final sample volumes from the treatment with both C_{18} (Strata-X) and amino sorbents were 2 mL. Therefore, the eluate from the SPE treatment was evaporated under nitrogen with mild heating (40 ^{o}C) to dryness, following by dissolution with methanol. The sample volume of 250 µL was separated from the final analytical sample for the analysis. The studies were performed with five replicates and with eight sequential analyses.

2.4 Optimization of the separation parameters

In this study, the PF-MEKC-UV method for corticosteroids (Sirén et al., 2015) was optimized for androgens and progesterone hormone separations. Testosterone and progesterone were used as the model compounds for adjusting the separation time. The injection pressure and time, concentration and pH of the electrolyte solution, capillary dimensions, applied electric field, temperature, and concentrations of SDS and sodium taurocholate in the micelle solution were optimized in our previous studies (Sirén and El Fellah, 2016a&b; Sirén et al., 2015).

The parameters in inorganic anions and cations were optimized for the Agilent instrument in this work. They were upgraded from (Hiissa et al., 1999; Harvanová and Bloom, 2015).

2.5 Preparation of standards and calibration solutions

The stock solutions of steroid hormones at 1000 µg/mL and inorganic cations and anions at 100 µg/mL were prepared in methanol and milli-Q water, and stored at +4 ˚C. The working solutions

were also prepared from the stocks. When stored in cold, the solutions were let to warm up to room temperature before use.

Concentration calibration for steroids (PF-MEKC-UV): The steroid concentrations were 0.5, 1, 2, 4, 6, 8, and 10 µg/mL for testosterone and androstenedione and 0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, 3, 4 and 5 µg/mL for progesterone. Otherwise, the measurements were at 0.5-10.0 µg/mL level.

Concentration calibration for inorganic ions (CZE – indirect-UV): Concentrations were from 0.1 µg/mL to 50 µg/mL depending on the ion.

3 Results and Discussion

The study was performed by capillary electrophoresis (CE) with UV detection and by liquid chromatography with high resolution mass spectrometry detection (UHPLC-HRMS). Orbitrap mass analyser for high-resolution and accurate-mass (HRAM) performance was used for confirming the identity of the separated unknown compounds in the samples. Mostly, partial-filling micellar electrokinetic capillary chromatography (PF-MEKC) technique was used, except for inorganic ions, which were studied with capillary zone electrophoresis (CZE). Analytical conditions were optimized by multiple analyses of the reference analytes.

3.1 Water samples

Totally, seventeen cold and hot water samples from households using the tap water for drinking and cooking were collected for our study. Twelve drinking water samples were from water purification plants serving the water for nearly 1.2 million-population in Southern Finland. They were more profoundly investigated. One of them, Seutula water was tap water from a well, located in a groundwater basin of a decentralization area. It was used as the reference. In addition, four drinking water samples from South-Eastern Finland were used as references for the analyses of inorganic compounds. They were tap water in Imatra (TW-I), drilled well water in Lappeenranta (DW-L), drilled well water in Lake Saimaa area (DW-LS), and tap water in Lappeenranta (TW-L). A cold tap water from a household in Vantaa (TW-V) was measured together as well.

Based on our earlier study done with influent and effluent water samples of wastewater purification plants, the samples of Helsinki plant contained progesterone at 128 ng/L and 5 ng/L level, respectively. Therefore, it was supposed that its concentration in drinking water is very low [29]. To study the real cases, various tap water samples were collected form the area and analysed.

3.2 Determination of the steroids with PF-MEKC

Capillary electrophoresis was chosen for the main method due to its good separation efficiency and the possibility to carry out online sample concentration for non-ionic compounds. The fastest mobility of the steroids belonged to androstenedione followed by botrydial, testosterone, and progesterone (Table 1). On the other hand, the anionic steroid glucuronides migrated even faster and therefore in the electropherograms the first peak origins from a glucuronide metabolite of estrogens. Interestingly, the migration order of the compounds in the PF-MEKC method was the same as in UHPLC-HRMS method, using reverse-phase stationary phase (Table 1). The UHPLC method was needed for characterizing the SPE concentrates obtained in sample treatment process. PF-MEKC was used for quantification of the steroids.

For quantitative measurements, screening of the profiles were used for the evaluation of the method concentration ranges for progesterone and the other steroids. Figure 1 a shows the profiles of blank and progesterone standard. Clean profiles without problems in identification can be seen. Figures 1 b-d show the profiles of cold and hot tap water extracts (original volume 2 L) of households in Helsinki. As is seen based on the standard profiles, progesterone is clearly detected in all water extracts. In addition, the extracts of tap water from other households (Myyrmäki and Porvoo, Figures 1 e and f) show that most probably the main steroid was progesterone. To convince the PF-MEKC analysis results the extracts were spiked with progesterone standard (2 µg/mL). The quantification was done with external standard calibrations (Table 2). The high concentration of botrydial (identified by UHPLC-orbitrap-MS) had an effect on the absolute migration times of the compounds moving later but slightly also on those passing the detection before it. The profile of our

reference water (well water from Seutula) was not as we expected since after SPE treatment, the extract was full of nutrients which can be seen in the Figure 1 g.

The concentrations of the steroids in the drinking water samples were determined with the calibration data compiled in Table 2. The LOD (signal-to-noise value 3) and LOQ values were experimentally measured using steroid standard mixtures. Those values were used as the method limits since solid phase extraction techniques allowed the analytes enrichment from 2 L into 250 µL. The EF value can be calculated from the process steps that reduce the sampling volume to 250 µL of the analytical concentrate.

Progesterone was detected at quite high concentration in all the water samples. The synthetic birth control hormone was quantified with five replicates to calculate the average values shown in Figure 2. The results of the cold drinking water samples indicated that the highest concentrations of progesterone were in Munkkiniemi (0.130 ng/L) and Seutula (0.135 ng/L). On the contrary, the highest hot water values were 0.191 ng/L and 0.175 ng/L in Munkkiniemi and Porvoo, respectively. Interestingly, the high values were supposed to be correlating with the pipelines, since lately the water pipes were renewed in Munkkiniemi and Porvoo. As a comparison, in the housing cooperative of Myyrmäki, the pipes were 15 years old correlating to the low steroid value. The average progesterone concentrations of the studied samples were below 0.20 ng/L.

As to the other compounds marked in the Figure 1, the first compound (peak 1) is attributed to estrogen glucuronide, the second to androstenedione (peak 2), and the fourth to testosterone (peak 4). These compounds were identified in all water samples except those from Seutula. Their concentrations were very low. The glucuronide conjugate of estrogen metabolite was 0.010 ng/L (max. value in the samples 1.3 µg/mL), androstenedione 0.030 ng/ L (max. value in the samples 3.7 µg/mL), and testosterone 0.015 ng/L (max. value in the samples 1.9 µg/mL). The results are comparable with the lately published data made with GC-HRMS, which shows that the typical

estrogen, 17β-estradiol (17β-E2) was detected in tap water samples at concentrations from 0.09 to 0.15 ng /L (Zacs et al., 2016).

3.3 Identification of mould and steroids by UHPLC-HRMS

Usually, the Finnish waters are soft, slightly acidic (pH < 7). Typically, water quality in Finland is affected by the soil and rock features and by shallowness and eutrophication (Hiisvirta, 2001). Flowing from plant to the consumer, the quality of water may deteriorate before used as tap water. Thus, it does not fulfil the quality demands of drinking water. It has been suggested that changes in pH can increase corrosion and improve the lift of precipitation adsorbed on the inner walls of the pipelines (Koponen, 2001). According to authorities, when tap water has odor and flavor problems the reason may be the PEX tubing. Therefore, chemical basic analysis of volatile organic compounds (VOCs) is required.

The tap water samples were from households, except the one that was from a well, which was not frequently used. For our purpose, mould-containing water was used the reference, since it was raw water and contained humus. It also represented the weak flowing and stagnant water sample (Figure 1). Interestingly, the concentrated tap water samples from pipelines contained even higher amounts of the mould marker, botrydial that is commonly metabolized from sesquiterpenes. That phytotoxic metabolite is secreted by the fungus *Botrytis cinerea*.

UHPLC-HRMS was used for identification of the extracts of Etu-Töölö and Myyrmäki. The mass-spectrometric study was made with samples from Strata-X sorbent in SPE, because the profiles made with the amino sorbents similar to those in Strata-X. Figure 3 presents the extracted ion chromatograms (EICs) of m/z 287.20035, m/z 289.21591 ions from standard samples and Myyrmäki water sample. Difference between theoretical and measured masses obtained by HRMS are small and well within commonly acceptable errors (i.e, ± 5 ppm). Based on the good agreement between the retention times and the accurate masses, the m/z 287.200 (RT 8.76 min) and m/z 289.215 (RT 9.13 min) found in Myyrmäki water sample, were attributed to androstenedione

($C_{19}H_{27}O_2$) and testosterone ($C_{19}H_{29}O_2$). Progesterone was also identified by HRMS in this work (m/z 315.23148, Δm = -0.38 ppm).

The migration and retention times of the botrydial are listed in Table 3. Its appearance in the electropherograms was true, since the blank water after SPE-treatment did not contain it. In addition, based on our earlier studies with the influent and the effluent water samples from wastewater pre-treatment plants, it was noticed that they did not contain botrydial. Furthermore, the extracts concentrated with amino sorbents contained only negligible amounts of botrydial, since Strata-X sorbent restrained it extremely effectively. Repeatability of the absolute migration times without correction was between 0.17 %-1.1 % (Table 3). That was assumed to ensure the repeatability of the analysis method. From the results compiled in Table 3 it can be calculated that the botrydial concentrations were 40, 59, 18, 0.4, 51, and 6.9 % higher in hot water than in cold water of Etu-Töölö, Kumpula, Munkkiniemi, Myyrmäki, Porvoo, and Seutula, respectively. However, its concentration was at high level in Munkkiniemi sample. In Myyrmäki sample, its concentration was similar in cold and hot water samples. To compare botrydial concentration in the reference water sample from the well, it can be calculated that its amount was 92, 88, 95, 94, and 95% higher for the above mentioned samples, in their respective order.

The identification of botrydial is supported by the MS and MS^2 spectra presented in Figure 4. Difference between theoretical and measured masses obtained by HRMS are small. According to the literature, similar study or characterization has not been reported.

3.4 Determination of inorganic anions and metals with CZE

Minerals in drinking water have shown to have impact on taste and importance on consumer health (Harvanová et al., 2014). Usually, the determination of the inorganic and heavy metal ions is accomplished using the methods of IC and ICP-AES, respectively. In our study, capillary electrophoresis was also used.

More than 100 years of research has focused on removing acute and chronic health threats to produce safe drinking water, but limited research has focused on the consequences of removing minerals that affect drinking water taste and health. The paper covers the human sense of taste, typical variations in the taste of drinking water, comparisons of global taste standards, the role of water chemistry, and future research needs for understanding consumer preference. Results of several consumptive tap and bottled water acceptability investigations, conducted by the authors, are presented.

The results showed that in all the tap water samples, main inorganic contaminants were Na (< 28 mg/L), K (< 4 mg/L), Ca (30 mg/L) and Mg (< 10 mg/L). Chloride was detected in water samples in concentrations < 50 mg/L, except in tap water in Imatra, in which the concentration was > 1000 mg/L. Average nitrate concentration of all samples were < 25 mg/L, except one individual sample having the ion in concentration > 65 mg/L. Concentration of heavy metal copper was < 5 mg/L.

Previous studies have shown that the mobility, bioavailability, and toxicological properties of metals are all very dependent on the chemical forms in which they occur in the nature (Whelton et al., 2007; Harvanová et al., 2014; Benotti et al., 2009; Finlex 2015).

4 Conclusions

Partial filling micellar electrokinetic chromatographic and capillary zone electrophoretic methods were applied to the determination of steroid hormones, mould indicator, and nutrients in household drinking water samples. Capillary electrophoresis was a suitable technique for comprehensive profiling of the sample extracts after solid phase extraction. With respect to the simple separation conditions and miniaturization benefits of the proposed CE technique, it was accurate method for quantification. UHPLC-HRMS method was needed for identification and matrix characterization to obtain accurate masses for the analytes and confirm their occurrence in the water samples.

Acknowledgements

Magnus Ehrnrooth foundation (S.E.) is acknowledged for the financial support.

The authors declare that they do not have competing financial interest concerning the project. They do not have any conflicts either.

5. References

Arditsoglou, A., Voutsa, D., 2010, Partitioning of EDCs in inland waters and wastewaters in the coastal area of Thessaloniki, Northern Greece. Environmental Science and Pollution Research (ESPR) 17, 529-538.

Aydin, E., Talinli, I., 2013, Analysis, occurrence and fate of commonly used pharmaceuticals and hormones in the Buyukcekmece Watershed, Turkey. Chemosphere 90, 2004–2012.

Benotti, M.J., Trenholm, R.A., Vanderford, B.J., Holady, C.J., Stanford, B.D., Snyder, S.A., 2009, Pharmaceuticals and Endocrine Disrupting Compounds in U.S. Drinking Water. Environmental Science and Technology 43, 597–603.

Barreiros, L., Queiroz, J. F., Magalhaes, L. M., Silva, A. M. T., Segundo, M. A., 2016, Analysis of 17-β-estradiol and 17-α-ethinylestradiol in biological and environmental matrices—a review. Microchemical Journal 126, 243–262.

Bevacqua, C.E., Rice, C.P., Torrents, A., Ramirez, M., 2011, Steroid hormones in biosolids and poultry litter: A comparison of potential environmental inputs. Sci. Total Environ. 409, 2120–2126.

Chang, H., Wu, S., Hu, J., Asami, M., Kunikane, S., 2008, Trace analysis of androgens and progestogens in environmental waters by ultra-performance liquid chromatography-electrospray tandem mass spectrometry. Journal of Chromatography A 1195 44–51.

ChemAxon, 2011, https://www.chemaxon.com/products/calculator-plugins/property-predictors/ (access in 7th September 2016).

DrugBank Version 4.3; http://www.drugbank.ca/ (access on 2016).

Duporté, G., Parshintsev, J., Barreira, L.M.F., Hartonen, K., Kulmala, M., Riekkola, M.-L., 2016, Nitrogen-Containing Low Volatile Compounds from Pinonaldehyde-Dimethylamine Reaction in the Atmosphere: A Laboratory and Field Study. Environmental Science and Technology 50, 4693–4700.

EPA, US Environmental Protection Agency, 2009, Drinking Water Regulations and Contaminants, National Primary Drinking Water Regulations, EPA 816-F-09-004.

EU 2013, European Union Directive 2013/39.

Finlex 1352/2015, Sosiaali- ja terveysministeriön asetus talousveden laatuvaatimuksista ja valvontatutkimuksista 17.11.2015.

Food Safety Regulations (408/52) 21/11/1952, Directive 89/397/ETY, Special edition in Finnish: Chapter 13, Volume 019, 47 – 50 (1989).

Harvanová, J., Bloom, L., 2015, Capillary Electrophoresis Technique for Metal Species Determination: A Review. Journal of Liquid Chromatography and Related Technologies 38, 371–380.

Hiissa, T., Sirén, H., Kotiaho, T., Snellman, M., Hautojärvi, A., 1999, Quantification of anions and cations in environmental water samples Measurements with capillary electrophoresis and indirect-UV detection. Journal of Chromatography A 853, 403–411.

Hiisvirta, L., 2001, Juomaveden laatu Suomessa. Ympäristöterveyden tutkimusohjelma (SYTTY), Household water seminar, Science Centre Heureka, Vantaa, Finland.

Kaunisto, T., 2002, Acceptance procedures for materials and products used in drinking water systems. The EAS process in Finland. VTT Research Notes 2180, ISBN 951–38–6111–2.

Koponen, A., 2001, Yhdenmukaistetut rakennustuotestandardit. SFS Information 33, 10.

Kuster, M., de Jose Lopez, A.M., Dolores H.M., Petrovic, M., Martin-Alonso, Barcelo, J.D., 2008, Analysis and occurrence of pharmaceuticals, estrogens, progestogens and polar pesticides in sewage treatment plant effluents, river water and drinking water in the Llobregat river basin (Barcelona, Spain). Journal of Hydrology, 358, 112-123.

Lubick, N., 2008, Drinking water contamination mapped, Nature, doi:10.1038/news.2008.1310,

Magi, E., Scapolla, C., M. Di Carro, M., Liscio, C., 2010, Determination of endocrine-disrupting compounds in drinking waters by fast liquid chromatography–tandem mass spectrometry. Journal of Mass Spectrometry 45, 1003–1011.

Ministry of Social Affairs and Health, Finland, Decree N 461, 2000, Demands for investigations for household waters quality and control. Helsinki 19.5.2000, 5 §.

Mons, M.N., Heringa, M.B., van Genderen, J., , Puijker, L.M., W. Brand, W., C.J. van Leeuwen, C.J., ,P. Stoks, P., van der Hoek, J.P., van der Kooij, D., 2013, Use of the Threshold of Toxicological Concern (TTC) approach for deriving target values for drinking water contaminants. Water Research 47, 1666–1678.

Mäkinen, R., 2008, Drinking water quality and network materials in Finland, Finnish Institute of Drinking Water/Prizztech Ltd: Publications of Finnish institute of drinking water 5.

Neale, P.A., Escher, B.I., Schafer, A.I., 2009, pH dependence of steroid hormone-organic matter interactions at environmental concentrations. Science of Total Environment 407, 1164–1173.

Oleszewski, J.M., Lozano, N., Haines, C., Rice, C.P., Ramirez, M., Torrents, A., 2013, Journal of Environmental Science and Health, Part A 48, 862–870.

Parliament of Finland, 2000, Decree of the Ministry of Social Affairs and Health Relating to the Quality and Monitoring of Water Intended for Human Consumption. Finnish Acts of Parliament 461/2000.

Pinedo, C., Moraga, J., Barua, J., González-Rodríguez, V.E., Aleu, J., Durán-Patrón, R., Macías-Sánchez, A.J., Hanson, J.R., Viaud, M., Hernández-Galán, R., Garrido, C., Collado, I.G., 2016,

Chemically Induced Cryptic Sesquiterpenoids and Expression of Sesquiterpene Cyclases in Botrytis cinerea Revealed New Sporogenic (+)-4-Epieremophil-9-en-11-ols. ACS Chemistry and Biology 11, 1391−1400.

Scott, P.D., Bartkow, M., Blockwell, S.J., Coleman, H.M., Khan, S.J., Lim, R., McDonald, J.A., Nice, H., Nugegoda, D., Pettigrove, V., Tremblay, L.A., Warne, M.S.J., Leusch, F.D.L., 2014, An assessment of endocrine activity in Australian rivers using chemical and in vitro analyses. Environmental Science and Pollution Research 21, 12951–12967.

Radjenović, J., Matošic, M., Mijatovic, I., Petrović, M., 2008, Membrane bioreactor (MBR) as an advanced wastewater treatment technology. Hdb Environmental Chemistry Vol. 5, Part S/2, 37–101. DOI 10.1007/698-5-093.

Rovio, S., Sirén, K., Sirén, H., Application of capillary electrophoresis to determine metal cations, anions, organic acids, and carbohydrates in some Pinot Noir red wines. Food Chemistry 124 (2011) 1194–1200.

Silva, C.P., Otero, M., Esteves, V., 2012, Processes for the elimination of estrogenic steroid hormones from water: a review. Environmental Pollution 165, 38-58.

Sirén, H., El Fellah, S., 2016 a, Steroids contents in waters of wastewater purification plants: determination with partial-filling micellar electrokinetic capillary chromatography and UV detection. International Journal of Environmental Analytical Chemistry 96, 1003-1021.

Sirén, H., El Fellah, S., 2016 b, Androgens, estrogens and progesterone concentrations in wastewater purification processes measured with capillary electrophoresis. Environmental Science and Pollution Research, ESPR-D-16-05532.

Sirén, H., El Fellah, S., Puolakka, A., Tilli, M., Turkia, H., 2015, Capillary Electrophoresis in Determination of Steroid Hormones in Environmental and Drinking Waters, in Capillary Electrophoresis (CE): Principles, Challenges and Applications (Ed. C. Reed), Chapter 9 - 3rd Quarter, 245-266, ISBN: 978-1-63483-122-2.

Sirén, H., Seppänen-Laakso, T., Oresic, M., 2008, Capillary electrophoresis with UV detection and mass spectrometry in method development for profiling metabolites of steroid hormone metabolism. Journal of Chromatography B 871, 375-382.

Sirén, H., Sirén, K., Sirén, J., 2015, Evaluation of organic and inorganic compounds levels of red wines processed from *Pinot Noir* grapes. Analytical Chemistry Research 3, 26–36.

Thuy, H.T.T., Nguyen, T.D., 2013, The potential environmental risks of pharmaceuticals in Vietnamese aquatic systems: case study of antibiotics and synthetic hormones. Environmental Science and Pollution Research 20, 8132–8140.

Velicu, M., Suri, R., 2009, Presence of steroid hormones and antibiotics in surface water of agricultural, suburban and mixed-use areas. Environmental Monitoring and Assessment 154, 349–359.

Whelton, A. J., Dietrich, A.M., Burlingame, G.A., Schechs, M., Duncan, S.E., 2007, Water science and technology. International Association on Water Pollution Research 55, 283-91.

WHO, Guidelines for drinking-water quality, 2011, Fourth edition, ISBN**:** 978-92-4-154815-1.

D. Zacs, D., Perkons, I., Bartkevics, V., 2016, Determination of steroidal oestrogens in tap water samples using solid-phase extraction on a molecularly imprinted polymer sorbent and quantification with gas chromatography-mass spectrometry (GC-MS). Environmental Monitoring and Assessment 188, 433-445.

Figure captions:

Figure 1. PF-MEKC electropherograms of the samples. Samples: a) Strata-X: blank (left) and progesterone standard (right) and extract of tap water b) in Etu-Töölö (left: cold water; right: hot water), c) in Kumpula (Department of Chemistry, University of Helsinki (left: cold water; right: hot water), d) in Munkkiniemi (left: cold water; right: hot water), e) in Myyrmäki Vantaa (left: cold water; right: hot water), f) in Porvoo (left: cold water; right: hot water) and g) from a well in Seutula (left: cold water; right: hot water). Experimental details are explained in Ch. 2.

Figure 2. Progesterone concentration in 12 samples used as drinking water. Cold and hot water samples were separately measured. The procedure of the sample preparation is describes in (Sirén, El Fellah, 2016b) with small modifications described in Experimental. The enhancement was compared with the concentrations in the original water samples. Quantification was made with PF-MEKC-UV. Concentrations are calculated with equations presented in Table 2.

Figure 3. Selected ion monitoring (SIM) chromatograms of a) m/z 287.200 ion (androstenedione standard) and m/z 289.215 ion (testosterone standard), b) Myyrmäki water sample and c) Identification of the compounds in the sample with the accurate masses corresponding their $[M+H]^+$ ions (m/z 287.20035 at -0.72670 ppm for androstenedione, $C_{19}H_{27}O_2$; m/z 289.21591 at -1.01932 ppm for testosterone, $C_{19}H_{29}O_2$).

Figure 4. Finding botrydial (grey peak) from the drinking water sample of Myyrmäki. The mass spectroscopic conditions are described in Experimental. **a)** PF-MEKC-UV electropherogram **b)** EIC of m/z 311.18 Da: UHPLC-HRMS chromatogram (left) and mass spectrum with SCAN(+) mode (right) **c)** MS^2 (+) m/z of 311.18 Da: UHPLC-HRMS EIC (left) and MS^2 (+) fragmentation pattern of m/z 311.18 Da (right).

Figure 5. Anions and cations in the drinking water samples. Samples: cold water in Etu-Töölö (CWT-ET), hot water in Etu-Töölö (HTW-ET), tap water in Imatra (TW-I), drilled well water in Lappeenranta (DW-L), drilled well water in Lake Saimaa (DW-LS), tap water in Lappeenranta (TW-L), cold tap water in Myyrmäki (CTW-M), hot tap water in Myyrmäki (HTW-M) and tap water in Myyrmäki Vantaa (TW-V).

Tables

Table 1. Structures of androstenedione, botrydial, testosterone, and progesterone. Theoretical and experimentally measured exact molar masses with migration times in CE and retention times in UHPLC. Accurate masses are detected with HRMS.

Table 2. Calibration data of steroids and inorganic ions.

Table 3. Identification of botrydial of the drinking water samples. Determination made with PF-MEKC. Cold and hot water samples were purified with C_{18} (Strata-X) nonpolar sorbent. Results with three repetitions.

Table 1

Click here to download Table: Table 1_water.docx

Table 1. Structures of androstenedione, botrydial, testosterone, and progesterone. Theoretical and experimentally measured exact molar masses with migration times in CE and retention times in UHPLC. Accurate masses are detected with HRMS.

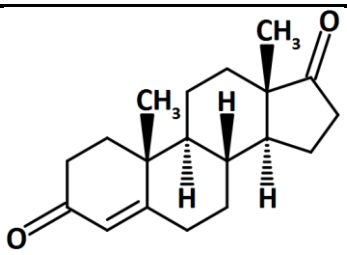
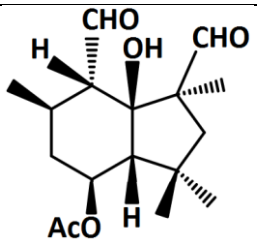
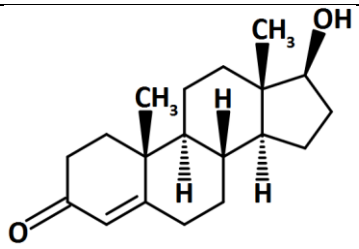
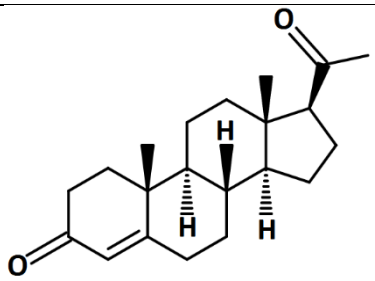
Compound	**Structure**	**Molar mass [g/mol]**	**$[M+H]^+$ of detected ion [m/z], (Δm)**	**Migration time [min]**	**Retention time [min]**
Androstenedione $C_{19}H_{26}O_2$		286.41	287.20035 (-0.73 ppm)	9.48	8.75
Botrydial $C_{17}H_{26}O_5$		310.385	311.18497 (-1.07 ppm)	10.57	8.87
Testosterone $C_{19}H_{28}O_2$		288.42	289.21609 (-1.02 ppm)	11.37	9.14
Progesterone $C_{21}H_{30}O_2$		314.462	315.23148 (0.38 ppm)	14.54	10.28

Table 2

Click here to download Table: Table 2_water.docx

Table 2. Calibration data of steroids and inorganic ions.

Electrolyte	Compound	Calibration range [µg/mL]	Linear equation	R^2	LOD [µg/mL]	LOQ [µg/mL]
PF-MEKC-UV*)	Progesterone	0.1 – 4	y = 1.6592x + 1.4526	0.948	0.10	0.30
	Estradiol-glucuronide	0.2 – 5	y = 1.16446x – 0.2115	0.970	0.20	0.60
	Androstenedione	0.5 – 8	y = 0.632x + 0.029	0.940	0.06	0.19
	Testosterone	0.5 – 8	y = 0.779x + 0.214	0.962	0.94	2.82
CE - Indirect-UV*)	Ammonium	0.1 – 25	y = 1.9665x + 0.9331	0.998	0.05	0.15
(Cations)	Copper	10 – 35	y = 3.0186x + 16.967	0.999	1.00	3.00
	Potassium	0.1 – 25	y = 1.1325x + 0.7301	0.998	0.05	0.15
	Calcium	0.1 – 25	y = 2.6928x + 5.7576	0.974	0.05	0.15
	Sodium	0.1 – 25	y = 0.4342x + 15.062	0.951	0.10	0.31
	Magnesium	0.1 – 25	y = 5.3278x + 3.9956	0.990	0.05	0.15
CE - Indirect-UV**)	Chloride	5 – 50	y = 0.9785x – 2.2476	0.992	0.10	0.30
(Anions)	Sulphate	5 – 50	y = 1.181*x* – 0.9429	0.998	0.10	0.30
	Nitrate	5 – 50	y = 0.6612x – 0.8557	0.990	0.10	0.30

*) polarity from anode to cathode; **) polarity from cathode to anode. Calibration of progesterone and estradiol-glucuronide: confidence range 95% and confidence factor 1.96.

Table 3
Click here to download Table: Table 3_water.docx

Table 3. Identification of botrydial of the drinking water samples. Determination made with PF-MEKC. Cold and hot water samples were purified with C_{18} (Strata-X) nonpolar sorbent. Results with three repetitions.

	Migration time [min]	Peak area [min*mAU]	Peak height [mAU]	Relative percentage [%]* $\frac{A_x}{A_{Seutula}} * 100\%$
Etu-Töölö, Cold				
Mean	10.026	376.085	37.786	1178
SD	0.060	1.580	0.333	
RSD%	0.60	0.42	0.88	
Etu-Töölö, Hot				
Mean	11.359	628.739	43.818	1833
SD	0.023	19.112	0.644	
RSD%	0.20	3.0	1.5	
Kumpula, Cold				
Mean	9.760	275.357	32.001	862
SD	0.018	3.983	0.277	
RSD%	0.18	1.4	0.86	
Kumpula, Hot				
Mean	10.629	664.129	49.157	1936
SD	0.018	14.990	0.197	
RSD%	0.17	2.3	0.40	
Munkkiniemi, Cold				
Mean	10.402	683.999	46.209	2142
SD	0.072	54.265	0.879	
RSD%	0.69	7.9	1.9	
Munkkiniemi, Hot				
Mean	12.186	831.530	46.786	2424
SD	0.095	27.303	0.704	
RSD%	0.78	3.3	1.5	
Myyrmäki, Cold				
Mean	9.266	495.302	43.798	1551
SD	0.074	2.105	0.142	
RSD%	0.79	0.43	0.32	
Myyrmäki, Hot				
Mean	10.027	497.355	42.772	1450
SD	0.028	5.491	0.236	
RSD%	0.28	1.1	0.55	
Porvoo, Cold				
Mean	10.301	652.074	49.356	2042
SD	0.052	21.196	0.072	
RSD%	0.51	3.3	0.15	
Porvoo, Hot				
Mean	11.798	1337.760	63.472	3900
SD	0.061	128.059	3.219	
RSD%	0.52	9.6	5.1	
Seutula, Cold				
Mean	10.692	31.938	8.957	100
SD	0.116	0.660	0.228	
RSD%	1.1	2.1	2.5	

Seutula, Hot				
Mean	11.585	34.303	8.380	100
SD	0.090	0.531	0.154	
RSD%	0.78	1.5	1.8	

*) Cold and hot water samples were calculated separately

Figure 1

Click here to download high resolution image

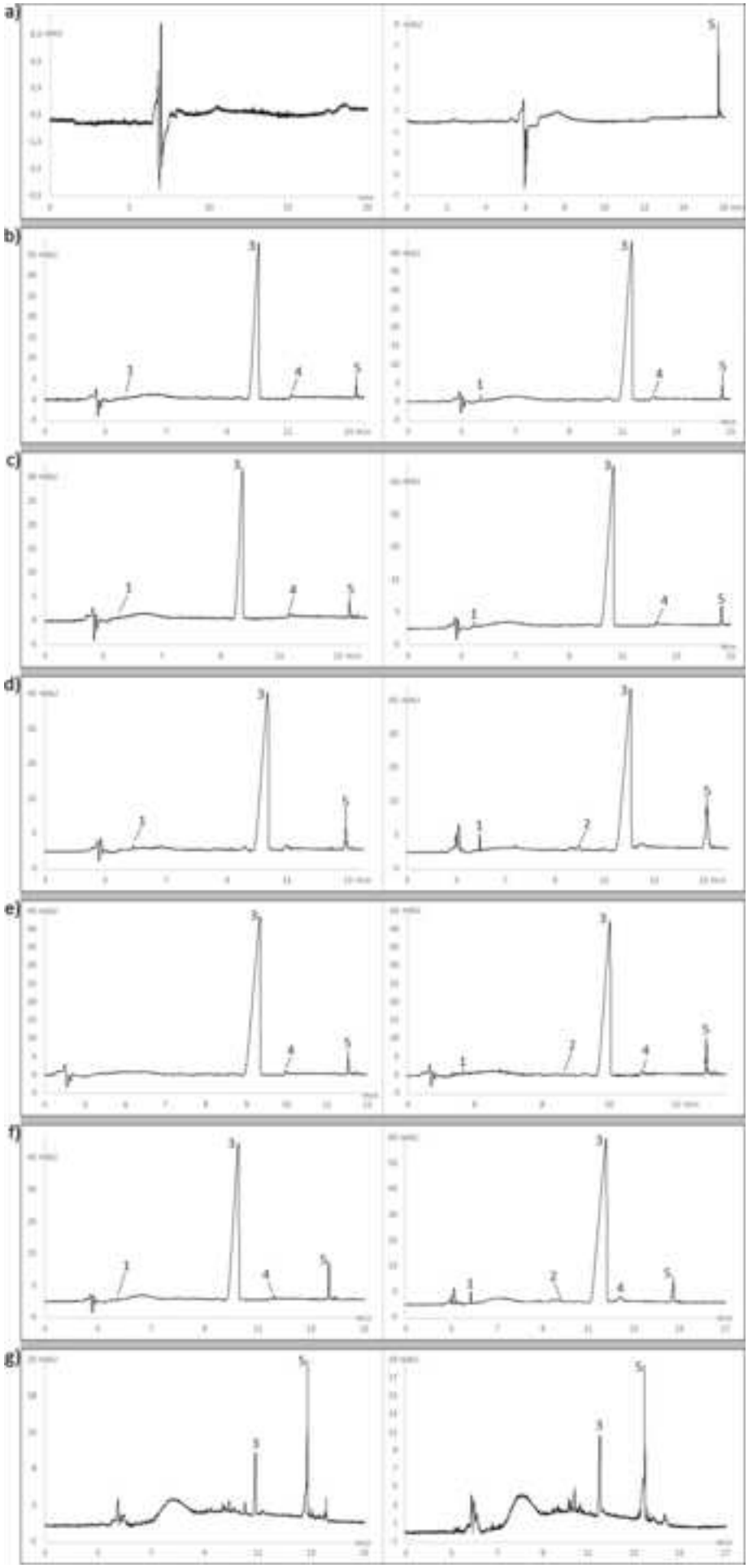


Figure 2

Click here to download high resolution image

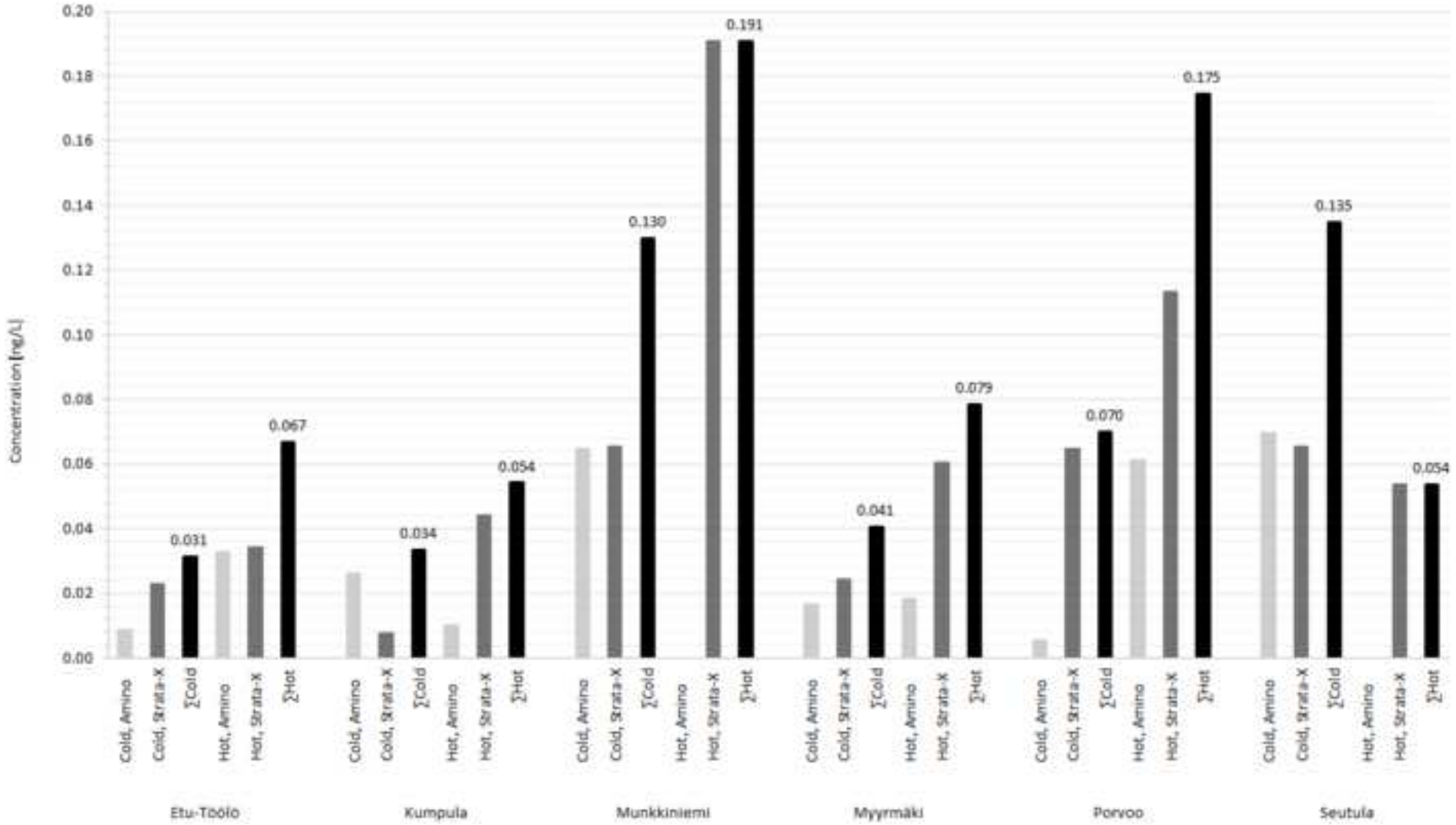


Figure 3

Click here to download high resolution image

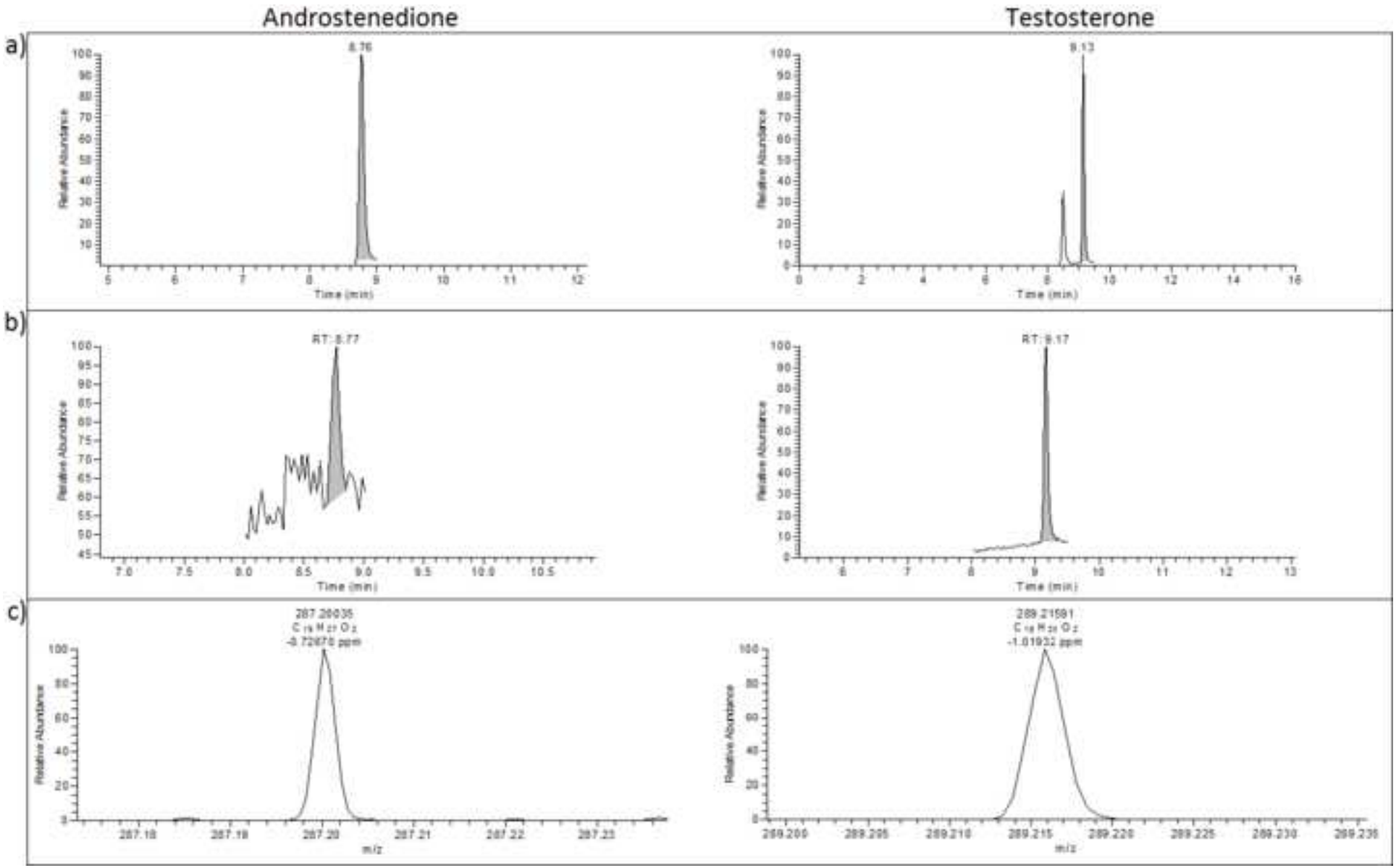


Figure 4

Click here to download high resolution image

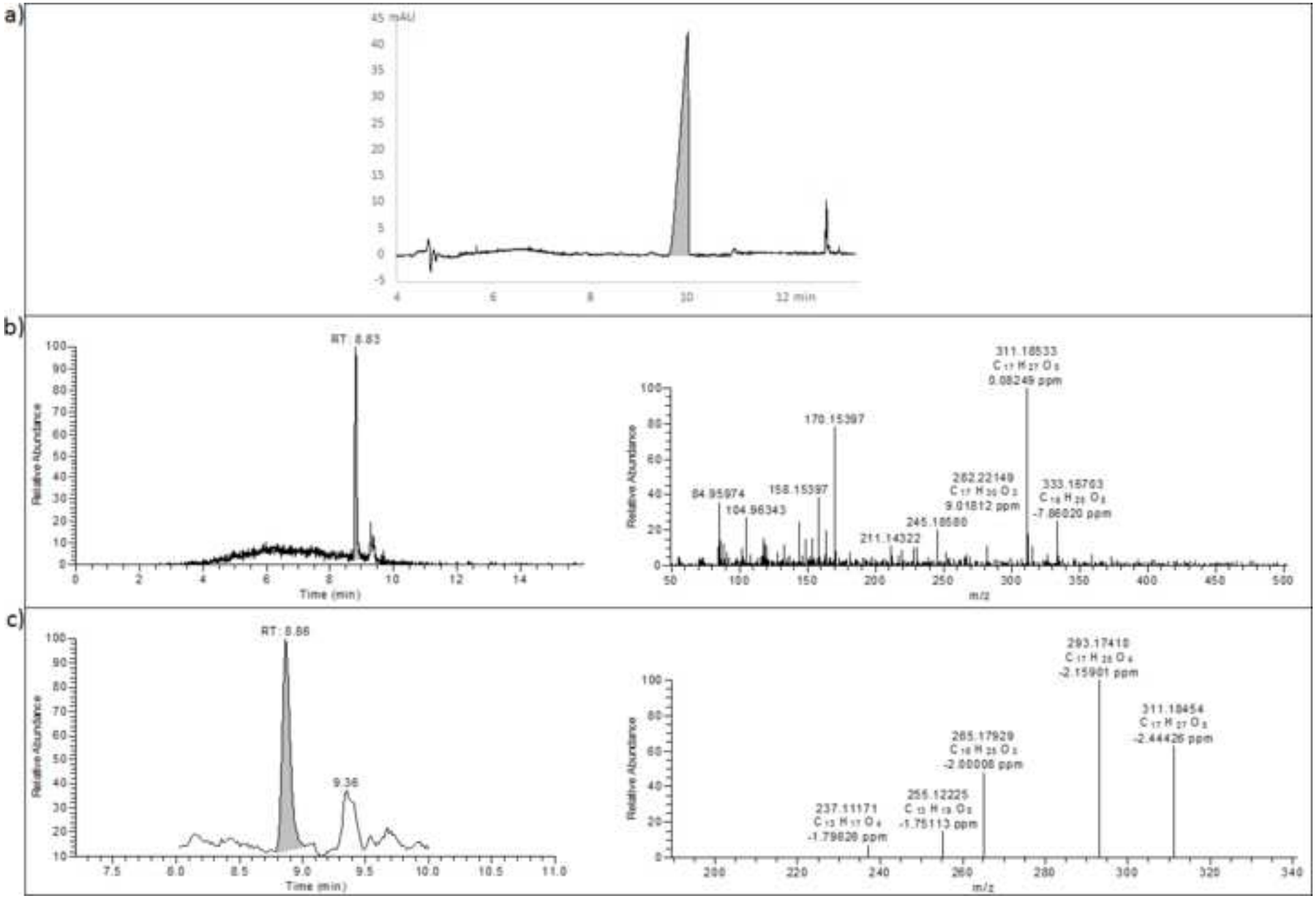


Figure 5

Click here to download high resolution image