Determination of Endocrine-Disrupting Chemicals in Drinking Water at Sub ng/L Levels Using the Agilent 6495 Triple Quadrupole Mass Spectrometer

Application Note

Authors

László Tölgyesi
Agilent Technologies Sales and Services GmbH & Co. KG, Chemical Analysis Group,
Hewlett-Packard-Str. 8, 76337 Waldbronn, Germany

Dan-Hui Dorothy Yang, Bernhard Wuest, and Anabel Fandino
Agilent Technologies, Inc.
5301 Stevens Creek Blvd,
Santa Clara, CA 95051-7201,
USA

Abstract

This Application Note demonstrates the precise, accurate, and robust determination of endocrine-disrupting chemicals (EDCs) in drinking water at low concentrations (ng/L) using the new Agilent 6495 Triple Quadrupole LC/MS system operated in dynamic multiple reaction monitoring (DMRM) and fast polarity (positive/negative) switching mode. The increased sensitivity of the instrument was used to streamline the analysis using direct large volume injection of tap water samples instead of more tedious and time-consuming sample enrichment by solid phase extraction (SPE) methods.

Agilent Technologies

Introduction

The presence of EDCs in the aquatic system has raised concerns about the aquatic environment and its relation to human health[1]. Excreted EDCs from humans and animals enter raw sewage and reach wastewater treatment plants either through direct discharge into the human effluent or agricultural runoffs. These compounds, if not removed during the various treatment processes (for example, oxidation and activated carbon), may lead to contamination of the drinking water system. EDCs in sufficient concentrations can interfere with the endocrine system and cause adverse health effects in an organism or its progeny. Recently, both estrogens and androgens have received considerable attention since they promote feminization and masculinization in fish[2].

EDC levels in municipal water supplies are regulated by several government agencies down to part-per-trillion (ppt) levels (EPA Method 539, EPA Method 1698)[3,4]. Such low concentrations (ng/L or pg/L) pose significant analytical challenges. Sample enrichment is often necessary using solid phase extraction (SPE) or liquid liquid extraction (LLE) where detection is performed using low to mid-range triple quadrupole mass spectrometers. Furthermore, both SPE and LLE require large sample quantities, high consumption of solvents, and laborious procedures[5].

This Application Note describes improvements to a previously published application note, and demonstrates how the increased sensitivity of the 6495 Triple Quadrupole LC/MS can be used to simplify the analytical workflow[5]. Several modifications of the triple quadrupole mass spectrometer have resulted in better analytical performance. Improvements include new front-end ion optics for increased precursor ion transmission, a newly designed curved and tapered collision cell providing enhanced MS/MS spectral fidelity, a new ion detector operating at dynode accelerating voltages up to 20 kV, and a new tune algorithm for enhanced speed and sensitivity. In addition, the 6495 Triple Quadrupole Mass Spectrometer uses the proven Agilent JetStream Ionization source in combination with a dual stage ion funnel and hexabore capillary for more efficient ion generation and sampling. The enhanced sensitivity enables large injection volumes of water samples so that sample enrichment is no longer required to meet the Limit of Detection (LOD) requirements at sub ng/L.

Experimental

Reagents and chemicals

All reagents and solvents were of HPLC-MS or analytical grade. Methanol and acetonitrile were purchased from Honeywell (Catalog number 230-4 and 015-4, respectively). Ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak). Ammonium fluoride was purchased from Fluka (338869-25 g), from which a 5 M stock solution was prepared by dissolving the appropriate amount of ammonium fluoride in Milli-Q water. The EPA 539 calibration stock standard was purchased from RESTEK (p/n 31998) containing the target hormone compounds in a single mix standard at the following concentrations: androstenedione (99.9 µg/mL), equilin (200.0 µg/mL), 17-β-estradiol (250.0 µg/mL), estriol (E3) (200.0 µg/mL), estrone (E1) (200.0 µg/mL), 17-α-ethinylestradiol (EE) (351.0 µg/mL), and testosterone (100.0 µg/mL). The chemical structures of these hormones are given in Figure 1.

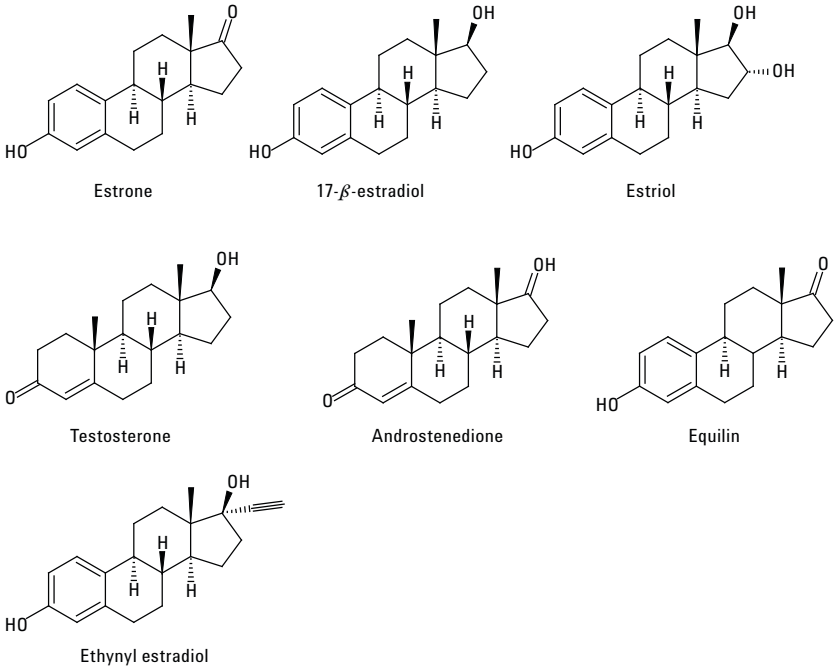

Figure 1. Structures of hormones.

Dilutions

The EPA 539 calibration stock standard was used to prepare the working standard in methanol:water (1:1 v/v) solution.

Compound concentrations in the working standard were as follows:

- Androstenedione: 10 ng/mL
- Equilin: 20 ng/mL
- 17-β-Estradiol (E2): 25 ng/mL
- Estriol (E3): 20 ng/mL
- Estrone (E1): 20 ng/mL
- 17-α-Ethynylestradiol (EE): 35 ng/mL
- Testosterone: 10 ng/mL.

Using the working standard, the calibration series detailed in Table 1 was prepared in tap water (source: Santa Clara, CA, USA).

Instrumentation

Chromatographic separation was carried out using an Agilent HPLC system consisting of an Agilent 1260 Binary Pump (G1312B), an Agilent 1260 Infinity Standard Autosampler (G1329B equipped with a 900-µL loop), sample cooler (G1330B), and an Agilent 1290 Infinity Thermostatted Column Compartment (G1316C). The HPLC system was coupled to an Agilent G6495 Triple Quadrupole Mass Spectrometer.

Agilent MassHunter Data Acquisition for Triple Quadruple Mass Spectrometer (version B 07.00) was used for data acquisition, while Agilent MassHunter Qualitative (version B 06.00) and Agilent MassHunter Quantitative software (version B 07.00) were used for data processing.

Method

A binary gradient method was used with 0.4 mM ammonium fluoride as Solvent A, and methanol:acetonitrile (1:1, v/v) as Solvent B. An Agilent Poroshell Phenyl Hexyl 2.1 × 100 mm, 2.7-µm column (p/n 695775-912) was used at a flow rate of 0.4 mL/min; the gradient is detailed in Table 2. The column temperature was maintained at 40 °C and injection volume was 900 µL (draw/eject speed: 1,000 µL/min; needle wash in vial: 100 % methanol). MS parameters and compound specific acquisition settings are presented in Tables 3 and 4, respectively. Cycle time was adjusted to 500 ms for the dynamic MRM acquisition mode, and the minimum and maximum dwell times were 34.1 and 249.1 ms.

MRM transitions for the target hormone compounds and associated collision energies were automatically determined in positive and negative mode using MassHunter Optimizer Software by flow injection analysis. Ion source parameters were optimized using the final chromatographic method with MassHunter Source Optimizer tool.

Table 1. Calibration series prepared in tap water (Santa Clara, CA).

Level	Testosterone (ng/L)	Androstenedione (ng/L)	Equilin (ng/L)	E3 (ng/L)	E1 (ng/L)	EE (ng/L)	E2 (ng/L)
6	10	10	20	20	20	35.1	25
5	5	5	10	10	10	17.55	12.5
4	1	1	2	2	2	3.51	2.5
3	0.5	0.5	1	1	1	1.76	1.25
2	0.2	0.2	0.4	0.4	0.4	0.7	0.5
1	0.1	0.1	0.2	0.2	0.2	0.35	0.25

Table 2. Gradient table.

Time (min)	% B
0.0	1
4.2	1
5.5	35
12.0	95
12.1	1
Stop time	16
Post time	6

Table 3. MS parameters – Positive and negative polarity.

Parameter	Value
Gas temperature	210 °C
Gas flow	15 L/min
Nebulizer	45 psi
Sheath gas temperature	375 °C
Sheath gas flow	12 L/min
Capillary voltage	3,500/4,000 ± V
Nozzle voltage	0/0 ± V
Delta EMV	250/250 ± V
HP RF voltage	190/190 ± V
LP RF voltage	80/100 ± V

Results and Discussion

Increased method performance

The 6495 Triple Quadrupole LC/MS design enhancements have demonstrated a significant increase in ion transmission. In addition, the detector design provides signal gains especially for negative ions. Figure 2 shows the response of 0.25 pg estrone on the 6495 Triple Quadrupole LC/MS. The increases in response comparing with a 6490 instrument, ranged from a factor of 2 to 5 for the seven hormones discussed in this study.

Instrument Detection Limit (IDL) and Lower Limit of Quantitation (LLOQ)

IDL refers to the minimum amount of analyte required to produce a signal that is statistically distinguishable from background noise with a given confidence level. This approach helps to avoid ambiguity related to the variation in the chemical noise and the different ways in which signal-to-noise (S/N) are determined. To test the sensitivity of the instrument and the feasibility of detecting hormones in drinking water at sub ppt level, target compounds were diluted in tap water using the working standard according to Table 1.

The mathematical formula for the IDL calculation is described below, where t corresponds to 99 % (1–α) confidence level at n–1 degrees of freedom (n = number of replicate injections, eight in this case), and RSD % is the relative standard deviation (precision) of signal response at the amount measured, from n replicate injections[6].

$$IDL_{LCMS} = t \cdot SD = t \cdot \left(\frac{RSD\%}{100}\right) \cdot amount\_measured$$

Table 4. DMRM parameters for the target compounds.

Compound name	Precursor ion	MS1 Res	Product ion	MS2 Res	RT	RT window	CE (V)	CAV (V)	Polarity
17-α-ethynylestradiol	295.2	Widest	145	Wide	12.4	1	47	2	–
17-α-ethynylestradiol	295.2	Widest	159	Wide			43	2	–
17-β-estradiol	271.2	Widest	183	Unit	12.2	1	47	2	–
17-β-estradiol	271.2	Widest	145	Unit			51	2	–
Androstenedione	287.2	Unit	108.9	Unit	12.7	1	26	2	+
Androstenedione	287.2	Unit	96.9	Unit			24	2	+
Equilin	267.1	Unit	265.1	Unit	12.5	1	28	4	–
Equilin	267.1	Unit	143.1	Unit			42	2	–
Estriol	287.2	Widest	171.2	Widest	10.8	1	44	2	–
Estriol	287.2	Widest	145	Widest			50	2	–
Estrone	269.2	Unit	145	Unit	12.7	1	43	2	–
Estrone	269.2	Unit	143	Unit			61	2	–
Testosterone	289.2	Unit	108.9	Unit	12.4	1	28	2	+
Testosterone	289.2	Unit	96.9	Unit			22	2	+

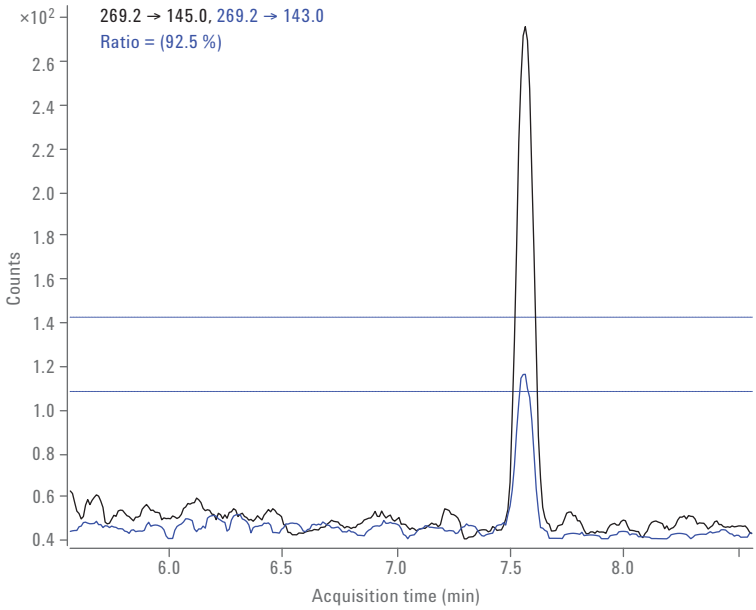

Figure 2. Signal response of the Agilent 6495 Triple Quadrupole LC/MS System (0.25 pg estrone on column).

Table 5 shows the RSD % and IDL as well as the spike concentrations at which the calculations were made.

Figure 3 shows an overlay of MRM chromatograms of the target compounds (quantifier and qualifier transitions) to illustrate the separation efficiency of the method.

LLOQ values were calculated (S/N > 10) for the quantifier (peak-to-peak), area RSD % < 20 and accuracy values within 80–120 %. The observed LLOQ values demonstrated very good correlation with the IDL values (data not shown) that ranged from 0.1 to 1.75 ng/L.

Table 5. The calculated IDL and RSD % values.

Compound name	Concentration (ng/L)	RSD%	IDL (ng/L)
17-α-Ethynylestradiol	1.8	14.8	0.78
17-β-estradiol	0.5	13.5	0.20
Androstenedione	0.2	4.3	0.03
Equilin	0.2	3.7	0.02
Estriol	1.0	5.6	0.17
Estrone	0.2	7.2	0.04
Testosterone	0.1	10.3	0.03

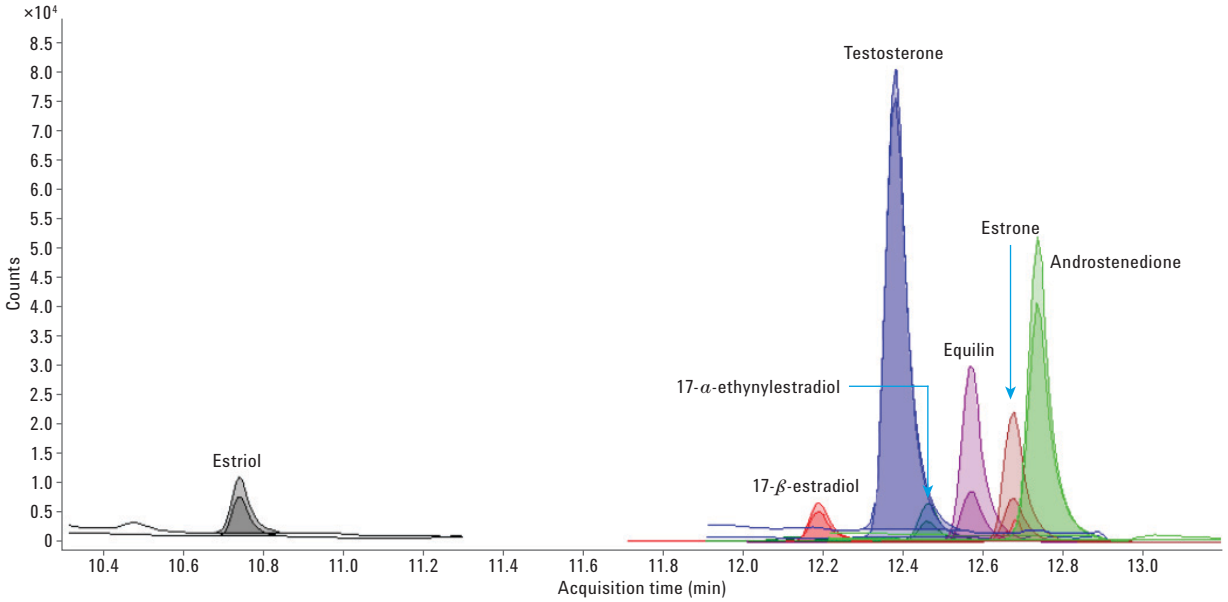

Figure 3. Chromatogram of calibration Standard 5 including all seven hormones (quantifier and qualifier transitions) in overlaid representation to illustrate the separation efficiency of the method.

The chromatograms at or close to the LLOQ levels for all seven hormones are shown in Figure 4.

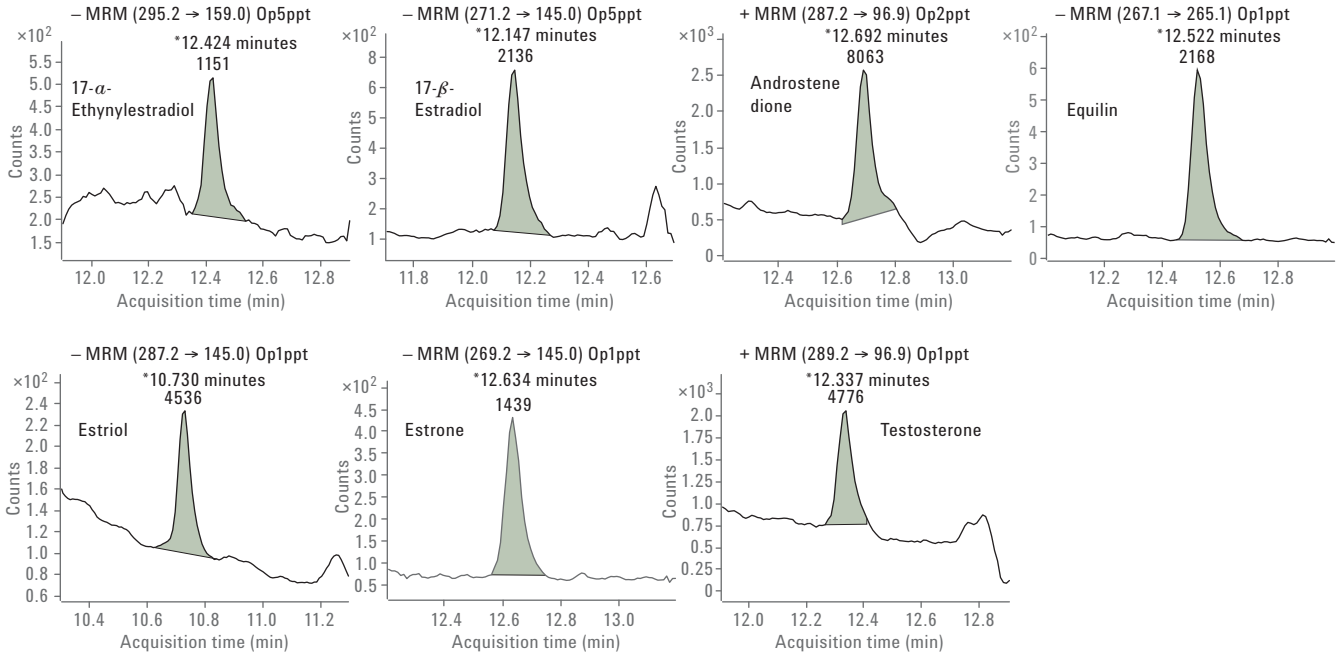

Figure 4. Extracted quantifier MRM transitions for all seven hormones at close to LLOQ level

Linearity

Linearity was assessed with spiked tap water samples covering a concentration range of 2 orders of magnitude (Table 1). Calibration curves for equilin and 17-β-estradiol are shown in Figure 5.

The equations of the linear fit and the corresponding correlation coefficients (R^2) for all target analytes are listed in Table 6. In each case, a weight factor of 1/x was applied.

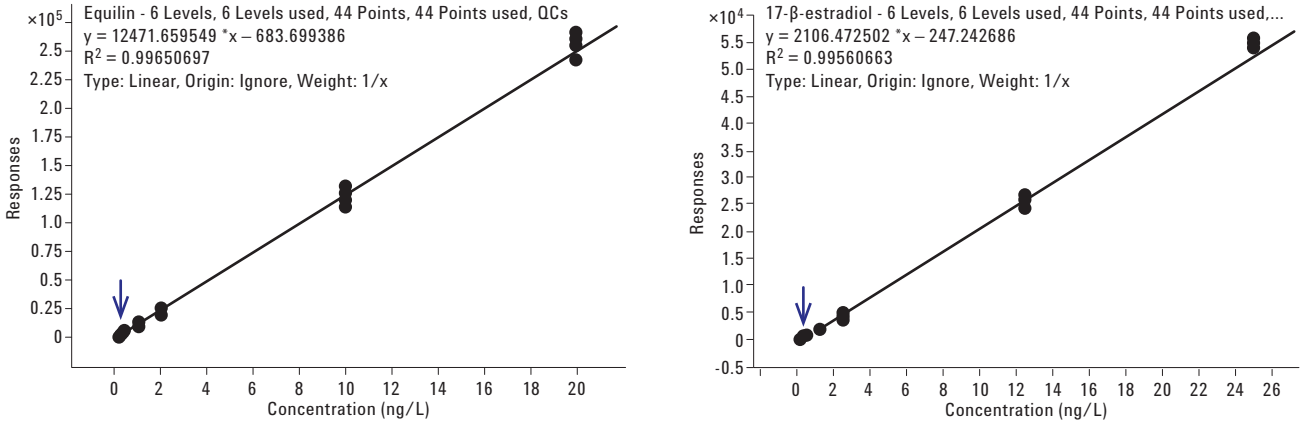

Figure 5. Calibration curves of equilin and 17-β-estradiol in spiked tap water samples.

Table 6. Linear regression parameters.

	Linear equation	R^2
17-α-Ethynylestradiol	y = 804.12x – 255.39	0.996
17-β-estradiol	y = 2106.47x – 247.24	0.996
Androstenedione	y = 43246.58x + 2273.65	0.995
Equilin	y = 12471.66x – 683.70	0.997
Estriol	y = 2257.79x + 277.07	0.997
Estrone	y = 8749.02x – 656.93	0.996
Testosterone	y = 65307.71x – 2825.52	0.994

Conclusion

A fast and simple LC/MS/MS method for the sensitive, precise and accurate quantitation of the hormones regulated by the EPA Method 539 has been developed using an Agilent 6495 Triple Quadrupole Mass Spectrometer. The enhanced instrument's sensitivity was demonstrated based on signal response precision with instrument detection limits (IDLs) ranging from 0.02 to 0.78 ng/L. Sub ng/L IDLs were achieved while using a streamlined analytical workflow with direct injection of tap water samples instead of time-consuming offline-solid phase extraction.

References

1. Flores-Valverde, A.M.; Horwood, J.; and Hill, E.M. Disruption of the steroid metabolome in fish caused by exposure to the environmental estrogen 17alpha-ethinylestradiol. *Environ. Sci. Technol.* **2010**, *44(9)* pp 3552-8.

2. Jenkins, R., *et al*., Identification of androstenedione in a river containing paper mill effluent. *Environ. Toxicology and Chem*. **2001**, *20(6)* pp 1325-1331.

3. EPA, Method 1698: Steroids and Hormones in Water, Soil, Sediment, and Biosolids by HRGC/HRMS. **2007**.

4. EPA, EPA Method 539 – Determination of Hormones in Drinking Water by Solid Phase Extraction (SPE) and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS), O.o.W.M. 140), Editor. **2010**.

5. Hindle, R., Improved Analysis of Trace Hormones in Drinking Water by LC/MS/MS (EPA 539) using the Agilent 6460 Triple Quadrupole LC/MS, *Agilent Technologies*, publication number 5991-2473EN, **2013**.

6. Parra, N.P; Taylor, L. Why Instrument Detection Limit (IDL) is a Better Metric for Determining The Sensitivity of Triple Quadrupole LC/MS Systems, *Agilent Technologies Technical Overview*, publication number 5991-4089EN, **2014**.

www.agilent.com/chem

Published in the USA, June 24, 2014
5991-4685EN

Agilent Technologies