

Determination of Endocrine Disrupting Chemicals in Drinking Water at Sub ng/L Levels using Direct Injection and 6495 Triple Quadrupole Mass Spectrometry

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Introduction

The presence of endocrine disrupting chemicals (EDCs) in the aquatic system has raised concerns about the aquatic environment and its impact on human health. In sufficient concentrations, these chemicals can interfere with the endocrine system causing adverse health effects in an organism or its progeny. As a result, EDC levels in municipal water supplies are regulated by several government agencies down to ng/L levels (EPA Method 539, EPA Method 1698) [1,2]. This requirement presents significant analytical challenges due to the EDC's low levels and wide variety of chemical structures.

In this work, we demonstrate how the increased sensitivity of the Agilent 6495 Triple Quadrupole LC/MS can be utilized to simplify the analytical workflow in tap water analysis. Several modifications on the hardware configuration have resulted in better analytical performance compared to previous high-end designs: new front end ion optics for increased precursor ion transmission, newly designed collision cell providing improved MS/MS spectral fidelity and a new ion detector operating at dynode accelerating voltages of up to 20 kV. In addition, the 6495 Triple Quadrupole MS uses the proven Agilent JetStream ionization source in combination with a dual stage ion funnel and hexapole capillary. The enhanced sensitivity enables large volume injections of water samples so that sample enrichment is no longer required to meet the Limit of Detection (LOD) requirements at sub ng/L.

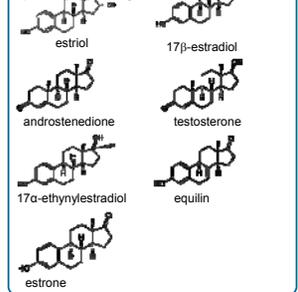


Figure 1: Chemical structures of EDCs

Experimental

Standard Preparation

The EPA 539 calibration stock standard was from RESTEK. The working solution was prepared in MeOH:H₂O (1:1) at 1:10,000 dilution from the EPA 539 stock solution before spiking into tap water covering the concentration range from 0.1 ng/L to 35 ng/L.

HPLC Method

The Agilent HPLC system consisted of an Agilent 1260 Binary Pump (G1312B), an Agilent 1260 Infinity Autosampler (G1326B with 900 µL loop), and an Agilent 1290 Infinity Thermostatted Column Compartment (G1316C).

Table 1: HPLC Method Parameters

HPLC parameters	Conditions
Column	Agilent Poroshell 120 Phenyl Hexyl 2.1 x 100 mm, 2.7 µm (P/N 695775-912)
Mobile Phase	A: H ₂ O + 0.4 mM ammonium fluoride B: Acetonitrile/Methanol (1:1, v/v)
Gradient	1% B for 4.2 min., 35% B at 5.5 min., 95% B at 12 min., 1% B at 12.1 min., Stop time at 16 min.
Flow rate	0.4 mL/min
Injection Volume	900 µL with draw and eject speed at 1000 µL/min

Mass Spectrometer Parameters

The 6495 Triple Quadrupole Mass Spectrometer was operated in dynamic multiple reaction monitoring (DMRM) and fast polarity (positive/negative) switching mode. The high energy dynode was operated at -10 kV in positive mode and 18 kV in negative mode. The MRM transitions and source parameters were optimized using the Agilent MassHunter Optimizer Software. The mass spectrometer parameters are listed in Table 2.

MS parameters	Setting
Drying gas temperature and flow	210°C and 15 L/min
Nebulizer pressure [psi]	45
Sheath gas temperature and flow	375°C and 12 L/min
Capillary voltage [V]	3500 (+)/4000 (-)
Nozzle voltage [V]	0 (+)/0 (-)
Delta EMV [V]	250 (+)/250 (-)
High pressure funnel RF voltage [V]	190 (+)/190 (-)
Low pressure funnel RF voltage [V]	80 (+)/100 (-)

Results and Discussion

Improved Performance of the 6495 QQQ Mass Spectrometer

The Agilent 6495 Triple Quadrupole LC/MS has a number of improvements to increase its performance and operational robustness. Enhanced sensitivity gives enhanced peak area response and improved peak area precision, which ultimately leads to lower detection limits compared to previous high-end designs. The increased MS sensitivity, if not required to lower the limit of detection, can be used to reduce sample preparation requirements as shown in this work for the direct injection of tap water samples. Figure 2 shows MRM chromatograms of quantifier transitions for the seven hormones at—or close to—their corresponding lower limit of quantitation (LLOQ) using the direct injection approach.

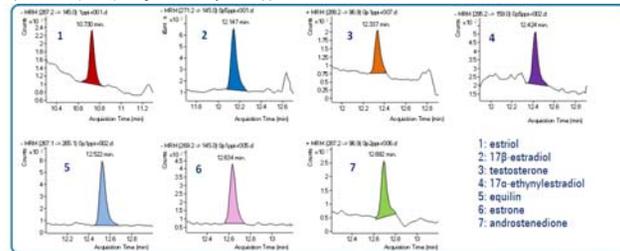


Figure 2: MRM chromatograms of quantifier transitions for all seven hormones at—or close to—their LLOQs using direct injection

Instrument Detection Limit (IDL) of EDCs in Tap Water

In order to test the sensitivity of the instrument and the feasibility of detecting hormones in drinking water at sub ppt levels, target compounds were diluted in tap water using the working standard and the instrument detection limit (IDL) was evaluated.

The definition of 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 avoid ambiguity related to the variation in the chemical noise and the different ways in which S/N ratios are determined. The mathematical formula for the IDL calculation is described below, where t corresponds to 95% (1- α) confidence level at $n-1$ degree of freedom (n = number of replicate injections, $n \geq 3$), and RSR is the relative standard deviation (RSD) of area response at the amount measured, from n replicate injections [3].

EDCs	Concentration [ng/L]	Area RSD [%]	IDL [ng/L], n=8
17 α -ethynylestradiol	1.75	14.8	0.78
17 β -estradiol	0.50	13.5	0.20
androstenedione	0.20	4.3	0.03
equilin	0.20	3.7	0.02
estrone	1.00	5.6	0.17
estrone	0.20	7.2	0.04
testosterone	0.10	10.3	0.03

Results and Discussion

Low limits of quantitation (LLOQs) of EDCs

Using the highly sensitive Agilent 6495 Triple Quadrupole LC/MS, EDCs can be detected down to low ppt levels using direct injection of tap water samples. LLOQ values were determined as the lowest concentration exhibiting S/N ratio (peak to peak) >10, area RSD [%] <20 and accuracy values within 80-120%. The observed LLOQ values demonstrated very good correlation with the IDL values (data not shown) and ranged from 0.1 to 1.75 ng/L depending on the compounds. Figure 2 shows the quantifier MRM chromatograms close to the LLOQ level for all seven hormones.

Figure 4 shows the chromatogram of EDCs and their eluting order in an overlaid representation to illustrate the separation efficiency of the chromatographic method.

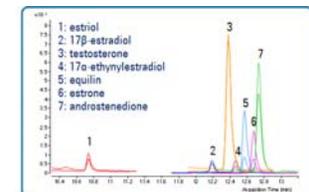


Figure 4: Overlaid MRM chromatograms (quantifier and qualifier transitions)

Linearity

Linearity was assessed with spiked tap water samples covering a concentration range of 2 orders of magnitude. The calibration curve for 17- β -estradiol is 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 4. In each case a weight factor of 1/x was applied.

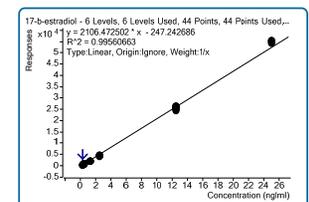


Figure 5: Calibration curve for 17- β -estradiol in spiked tap water

Table 4: Linear regression parameters

EDCs	Linear Equation	R ²
17 α -Ethynylestradiol	$y = 804.12x - 255.59$	0.999
17 β -estradiol	$y = 2108.47x - 247.24$	0.999
Androstenedione	$y = 43246.58x + 2213.85$	0.995
Equilin	$y = 12411.06x - 083.00$	0.993
Estrone	$y = 8740.07x + 166.93$	0.996
Testosterone	$y = 65307.71x - 2629.52$	0.994

Conclusions

- The increased sensitivity of the 6495 Triple Quadrupole enabled a streamlined analytical workflow with direct injection of tap water samples instead of time-consuming sample preparation procedures such as offline solid phase extraction.
- Quantitative performance based on signal response precision was used to demonstrate the instrument's sensitivity with IDLs ranging from 0.02 to 0.78 ng/L.
- Assay performance (correlation coefficients for calibration curves, area precision and accuracy) were excellent and within current regulatory

References

- [1] EPA, Method 1698: Steroids and Hormones in Water, Soil, Sediment, and Biotools by HRGC/HRMS, 2007.
- [2] 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.J.W.M. 140), Editor, 2010.
- [3] Parra N P, Taylor L (2014) Why Instrument Detection Limit (IDL) is a Better Metric for Determining the Sensitivity of Triple Quadrupole