



## **Sample Concentration and Analysis of Human Hormones in Drinking Water**

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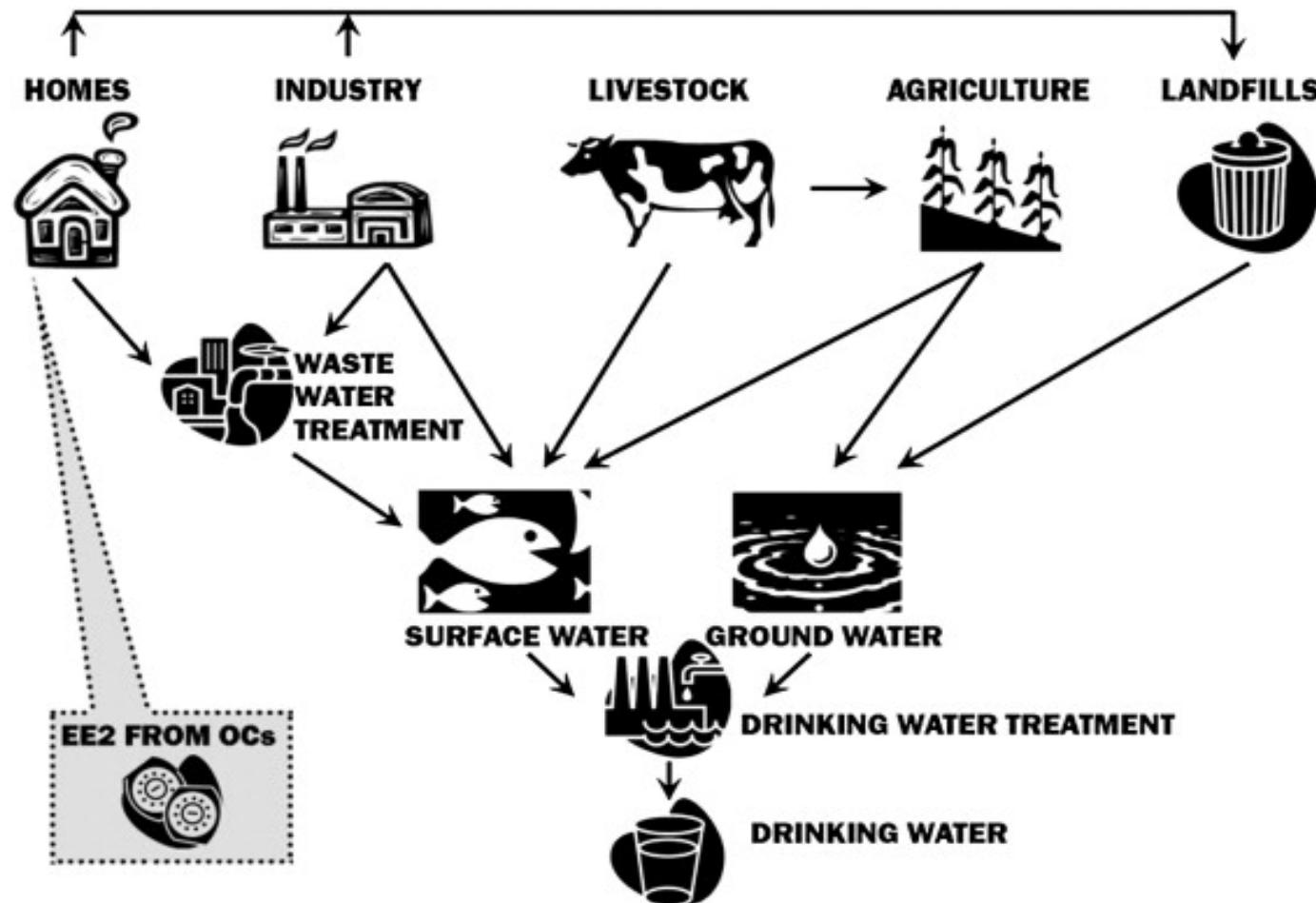
# Pharmaceutical Residues in Water Supplies

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- In 2008 the U.S. Geological Survey (USGS) tested tap water in nine states across the country and found 85 man-made chemicals, including some medications
- Many research centers and news outlets have reported traces of various pharmaceuticals in drinking water supplies, including:
  - Antibiotics
  - Anticonvulsants
  - Mood stabilizers
  - **Synthetic hormones (oral contraceptives)**



# How Do Hormones Get into Drinking Water?



Wise A, O'Brien K, Woodruff T.; *Environ Sci Tech*. 2011;1:51–60

# Health Risks of Hormones in Water Supplies

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- Hormones in water supplies are at very low concentration (ppb or ppt levels)
- Even extremely diluted concentrations of hormone residues harm aquatic food sources, such as freshwater fish
- Long-term consequences
  - Cancer: a number of types of cancers are hormone responsive
  - Male infertility:
    - Links have been established between reduced sperm count in fish and estrogen in water
    - Studies in humans are on-going in EU and the US
  - Obesity: weight-gain has been linked to rising estrogen levels
- “Stew Effect”
  - Potential interactions between trace amounts of chemicals in water

# U.S. EPA Method 539

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- Determination of Hormones in Drinking Water by Solid-Phase Extraction (SPE) and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS)
- On April 16, 2012 the EPA signed the third Unregulated Contaminant Monitoring Rule (UCMR 3)
  - Requires monitoring for 30 contaminants using EPA and/or consensus organization analytical methods during 2013-2015.
  - EPA Method 539 is included in UCMR 3:

Screening Survey	
7 Hormones using EPA Method 539 (LC/MS/MS): <sup>11</sup>	
17-β-estradiol .....	estrone.
17-α-ethynodiol (ethynodiol) .....	testosterone.
estriol (16-α-hydroxy-17-β-estradiol) .....	4-androstene-3,17-dione.
equilin.	

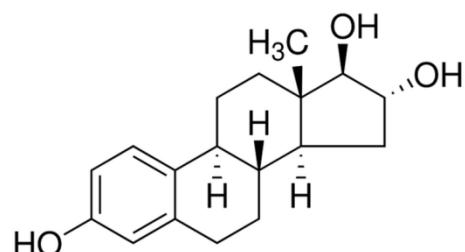


# Hormones Monitored: EPA Method 539

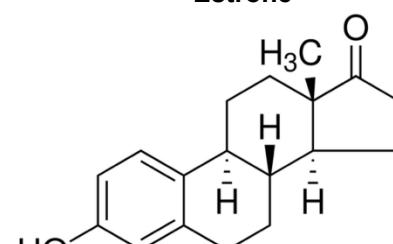
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## Estrogens

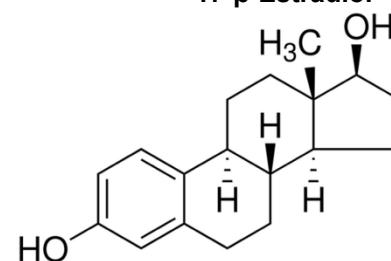
Estriol



Estrone

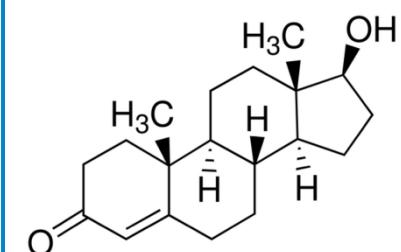


17- $\beta$ -Estradiol

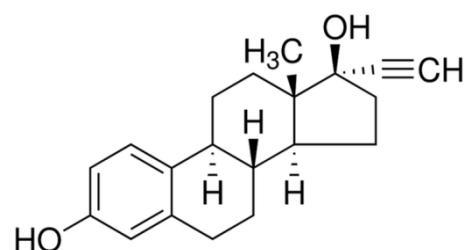


## Androgens

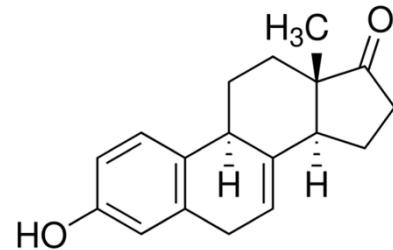
Testosterone



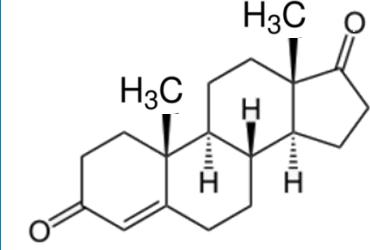
17- $\alpha$ -Ethynylestradiol



Equilin

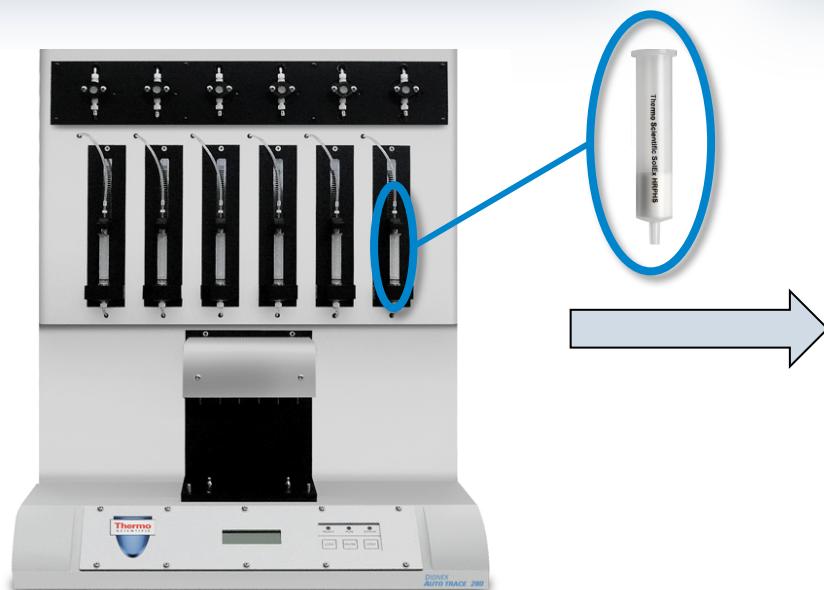


Androstenedione



# Hormone Analysis: Instrumentation Used

- SPE
  - Thermo Scientific™ Dionex™ AutoTrace™ 280 Solid-Phase Extraction Instrument
    - Thermo Scientific™ Dionex™ SolEx™ SPE HRPHS Cartridges



- LC-MS/MS
  - Thermo Scientific™ Dionex™ UltiMate™ 3000 LC system and Thermo Scientific™ TSQ Quantiva™ Triple Quadrupole Mass Spectrometer



# Dionex AutoTrace 280 Solid-Phase Extraction Instrument

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- Automated SPE of large-volume aqueous or water samples
  - 20 mL to 4 L sample volume
  - Drinking water and ground water
  - Positive pressure
- Sample Prep for Organic Analytes
  - Priority organic pollutants, personal care products, and endocrine disruptors
- Automated SPE
  - Automate all SPE steps: condition, load, rinse, and elute
  - Use normal or reversed-phase cartridges and disks
    - 1, 3, and 6 mL SPE cartridges



***Saves time and solvent, ensures reproducibility and analytical precision***

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# SPE Cartridges

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- Dionex SolEx cartridges
  - Silica-, Carbon-, Polymer-based
  - HRPHS
    - Neutral resin comprised of high-surface area, divinylbenzene-based particle
    - Hydrophilic, reversed-phase properties
    - High recovery of hydrophobic targets
  - 6 mL with 200 mg resin

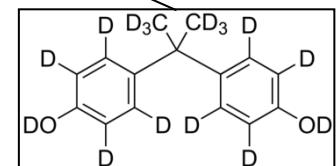


# Sample Concentration: Dionex AutoTrace 280 SPE Instrument



1 L Water

Sodium Omadine (Biocide)  
Sodium Thiosulfate (Dechlorination)  
Bisphenol A-d<sub>16</sub> (MS Surrogate)  
+ Hormones



<b>Condition</b>	MeOH; Water; N <sub>2</sub>
<b>Load</b>	10 mL/min
<b>Rinse</b>	10 mL 15% MeOH
<b>Dry</b>	N <sub>2</sub> , 10 min
<b>Elute</b>	2 x 3 mL + 4 mL MeOH

Concentrate  
to dryness  
(N<sub>2</sub>, 45 °C)

+ 1 mL  
50% MeOH

Estriol-d<sub>2</sub>  
<sup>13</sup>C<sub>6</sub>-Estradiol  
<sup>13</sup>C<sub>2</sub>-Ethynelestradiol  
Testosterone-d<sub>3</sub>

MS Internal Standards

# HPLC System

- UltiMate 3000 RSLC system



- Degasser
- Dual-gradient Pump
- Thermostatted Autosampler
- Thermostatted Column Compartment
- Diode Array Detector



Thermo Scientific™ Acclaim™  
Rapid Separation LC (RSLC)  
Polar Advantage II column  

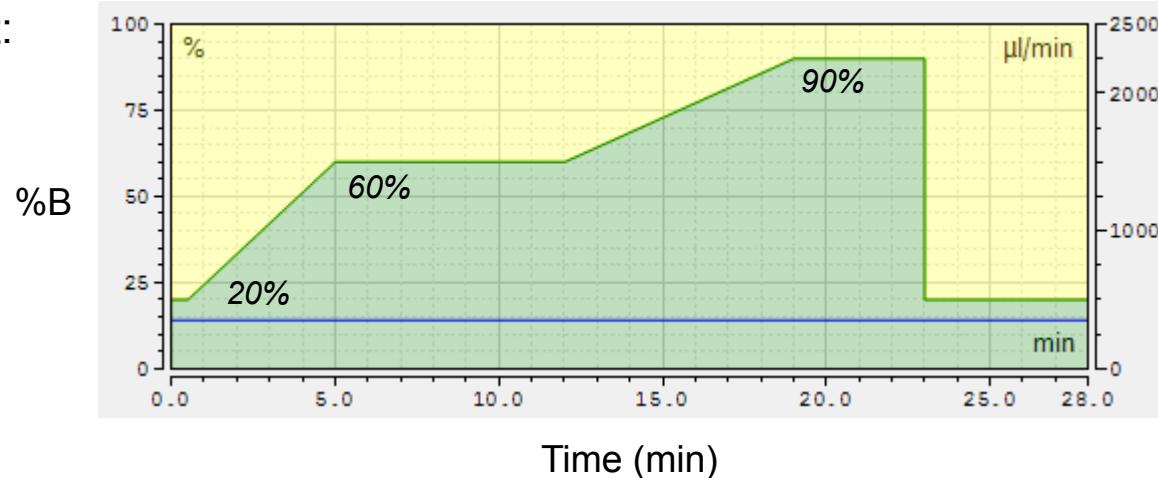
- 2.2 µm particle size, 2.1 × 150 mm
- pH 1.5–10

# HPLC Conditions

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Injection volume	50 µL
Column temperature	25 °C
Mobile phase A	Water + 0.02% ammonium hydroxide
Mobile phase B	50:50 MeOH:Acetonitrile + 0.02% ammonium hydroxide
Flow rate	350 µL/min

Gradient:



# TSQ Quantiva Mass Spectrometer

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- Attogram sensitivity
  - Thermo Scientific™ Active Ion Management (AIM™)
  - Ultrafast selected-reaction monitoring (SRM)
- Enhanced usability
  - Intuitive drag-and-drop method editor software
  - Thermo Scientific™ Ion Max NG™ ion source
    - Automatic gas and electrical connections
- Increased productivity
  - Application specific software
    - Thermo Scientific™ TraceFinder™ Software provides an extensive database of SRM parameters



# Mass Spectrometry Conditions

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Ion source	HESI III
Spray voltage	3000 V (-) 3250 V (+)
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	8 arbitrary units
Sweep gas pressure	1 arbitrary units
Ion transfer capillary temperature	350 °C
Vaporizer temperature	365 °C
Scan type	SRM
Q1 and Q3 peak width (FWHM)	0.7 Da
Collision gas and pressure	Argon at 1.5 mTorr

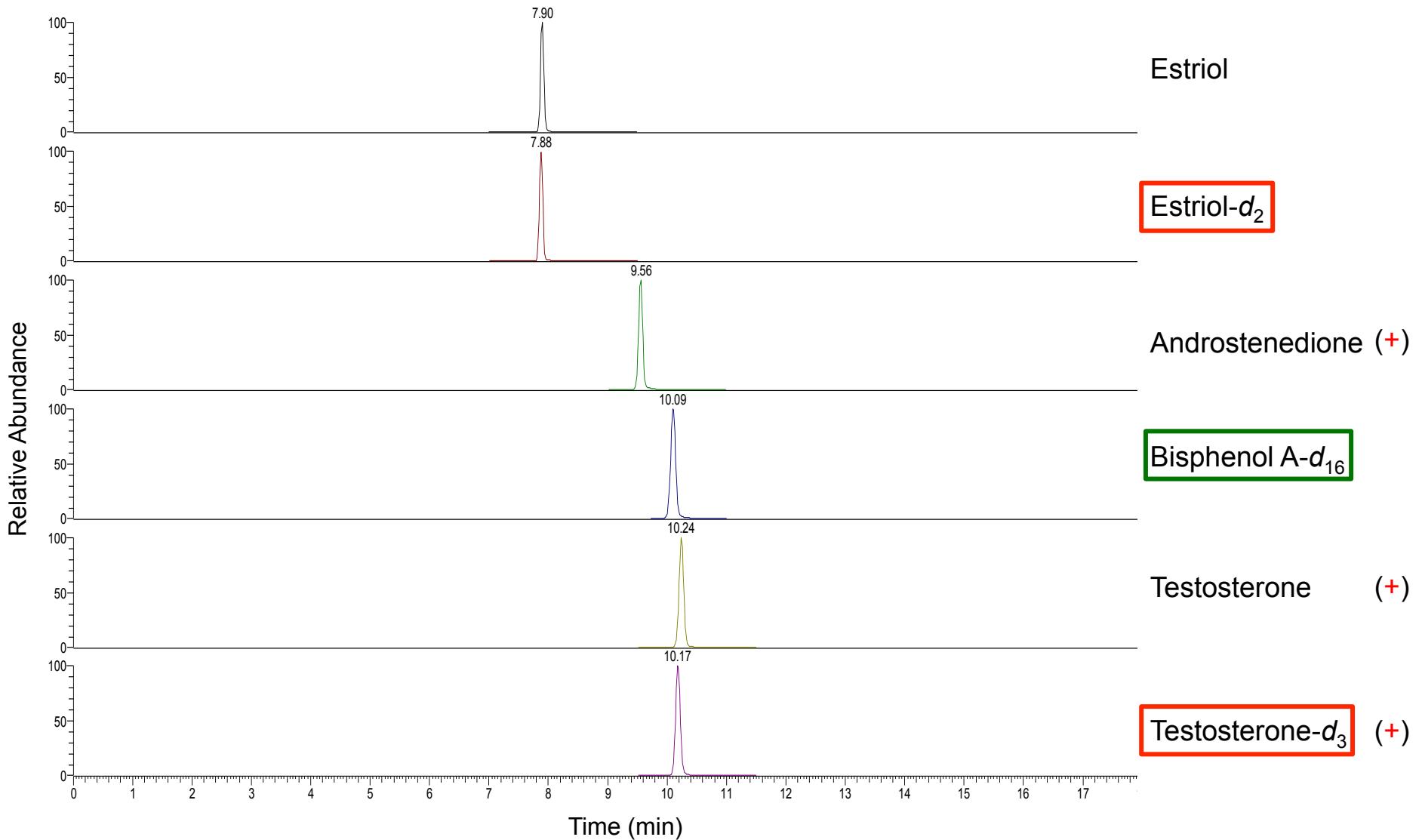
# ESI-MS/MS Mass Transitions

Compound	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V), RF-lens (V)*	Polarity
Estriol	287.2	145.0	44, 142	-
Estriol-d <sub>2</sub>	289.2	147.1	42, 113	-
Bisphenol A-d <sub>16</sub>	241.3	223.1	20, 84	-
Equilin	267.2	143.0	35, 106	-
Estrone	269.1	145.1	40, 101	-
<sup>13</sup> C <sub>2</sub> -Ethynylestradiol	297.2	145.1	48, 128	-
Ethynylestradiol	295.2	145.2	42, 106	-
17-β-Estradiol	271.2	145.3	46, 129	-
<sup>13</sup> C <sub>6</sub> -Estradiol	277.1	145.3	38, 139	-
Androstenedione	287.1	97.1	23, 97	+
Testosterone	289.2	97.2	25, 94	+
Testosterone-d <sub>3</sub>	292.2	97.1	24, 90	+

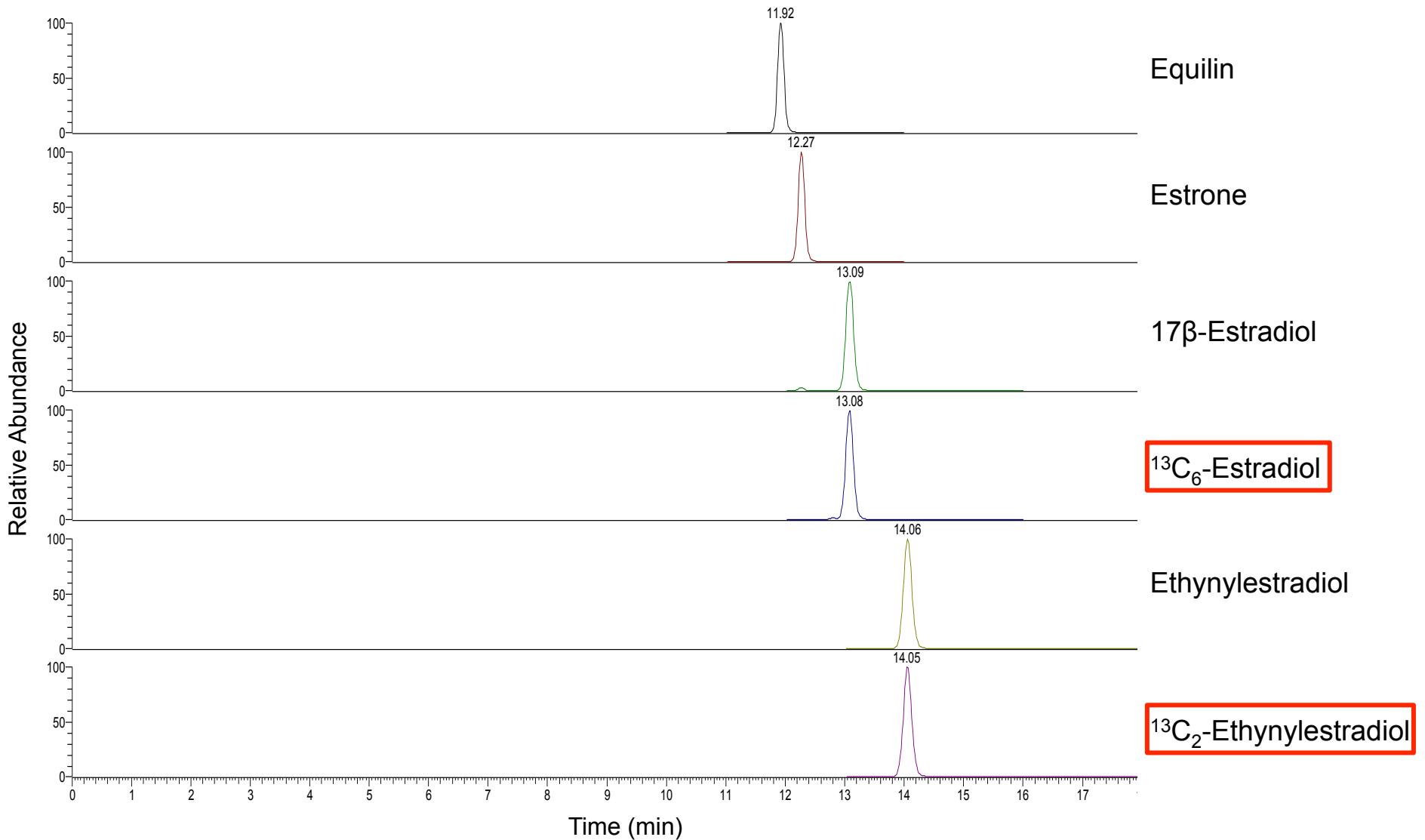
\*All parameters for SRM transitions should be optimized for each instrument

Red = MS internal standard; Green = MS surrogate standard

# Analyte Chromatograms



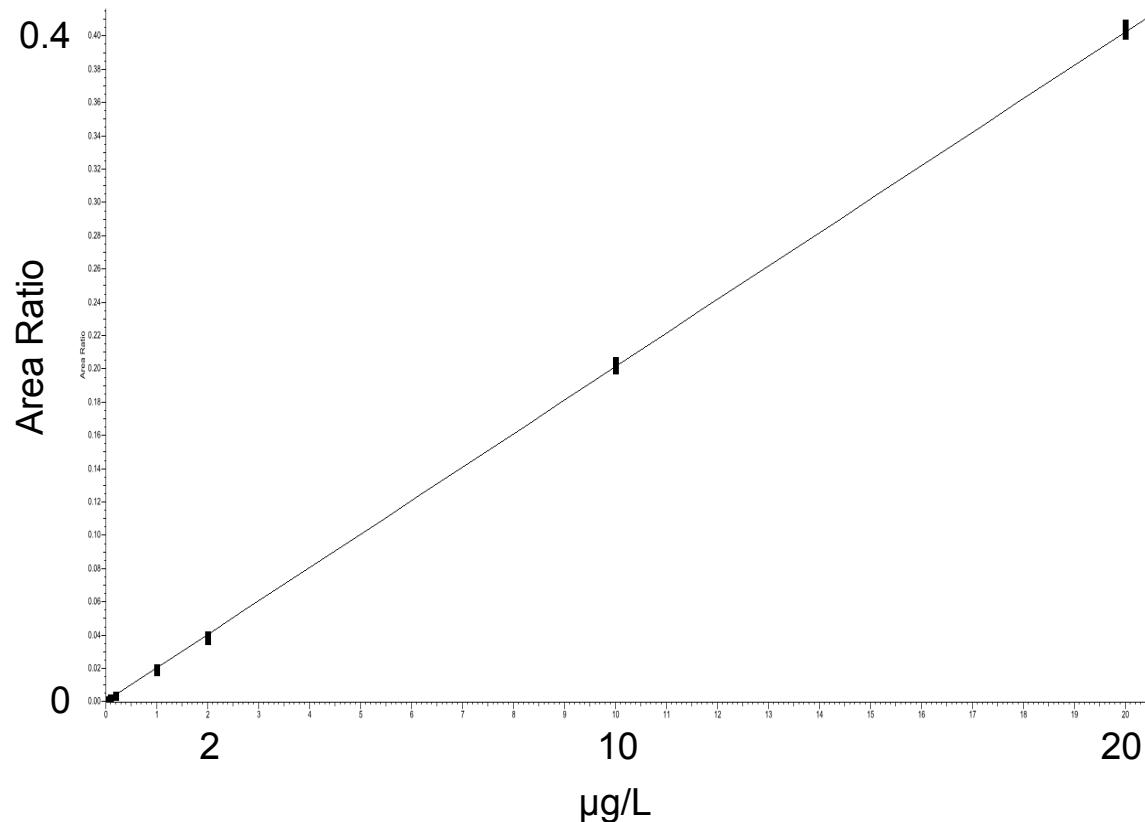
# Analyte Chromatograms



# Standard Curve Linearity

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- Standard Dilutions
  - Hormones + Surrogate + Internal Standards



Androstenedione:  
 $R^2 = 0.9996$   
0.02–20 μg/L

# Recovery of Hormones from Fortified Drinking Water

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Hormone	Spiked (ng/L)	Measured (ng/L)*	% Recovery*
Estriol	4	4.0	101
Estrone	4	4.8	121
17-β-Estradiol	5	4.9	98.1
17-α-Ethynodiolide	7	8.1	116
Androstenedione	2	2.0	98.9
Testosterone	2	2.0	99.0
Equilin	4	4.3	107

\*Triplicate injections of duplicate concentrations

*Recovery ranged from 98-121 %*

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# Detection Limits

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Hormone	Fortified (ng/L)	EPA (ng/L)	Calculated (ng/L)*
Estriol	0.20	0.24	0.05
Estrone	0.20	0.19	0.12
17-β-Estradiol	0.25	0.39	0.11
17-α-Ethyneestradiol	0.35	0.33	0.07
Androstenedione	0.10	0.20	0.10
Testosterone	0.10	0.04	0.01
Equilin	0.20	2.90	0.04

\*Triplicate injections of 7 replicate concentrations

*MDLs ranged from 0.01–0.12 ng/L*

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# Conclusion

- Solid-Phase Extraction -> HPLC -> Triple Quadrupole MS



Dionex AutoTrace 280 SPE  
Instrument



UltiMate 3000 LC  
System      TSQ Quantiva MS  
System

- Well differentiated MS peaks
- Hormones in drinking water concentrated with ~100% recovery
- Low ng/L MDLs

# Thermo Scientific Dionex Sample Prep Product Line

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Thermo Scientific™ Dionex™ ASE™  
150/350 Accelerated Solvent Extractor  
Systems



Dionex AutoTrace 280  
Solid-Phase Extraction  
Instrument



Dionex SolEx  
SPE Cartridges



Thermo Scientific™  
Dionex™ Rocket™  
Evaporator System

# Thank you!

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