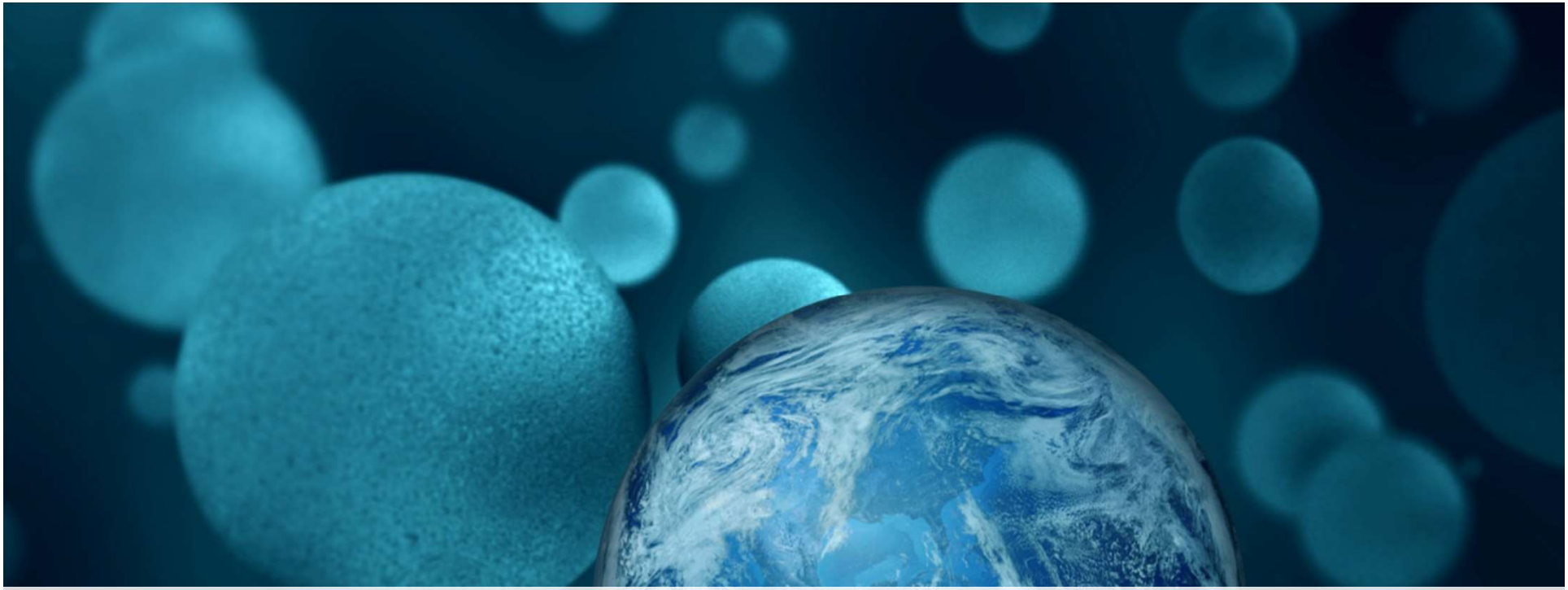

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Expanded Analysis of Human Hormones in Drinking Water Using Solid-Phase Extraction and Liquid Chromatography Tandem Mass Spectrometry

Carl Fisher, Claudia Martins, Ed George, and Pranathi Perati
Thermo Fisher Scientific

Pharmaceutical Residues in Water Supplies

- 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)**

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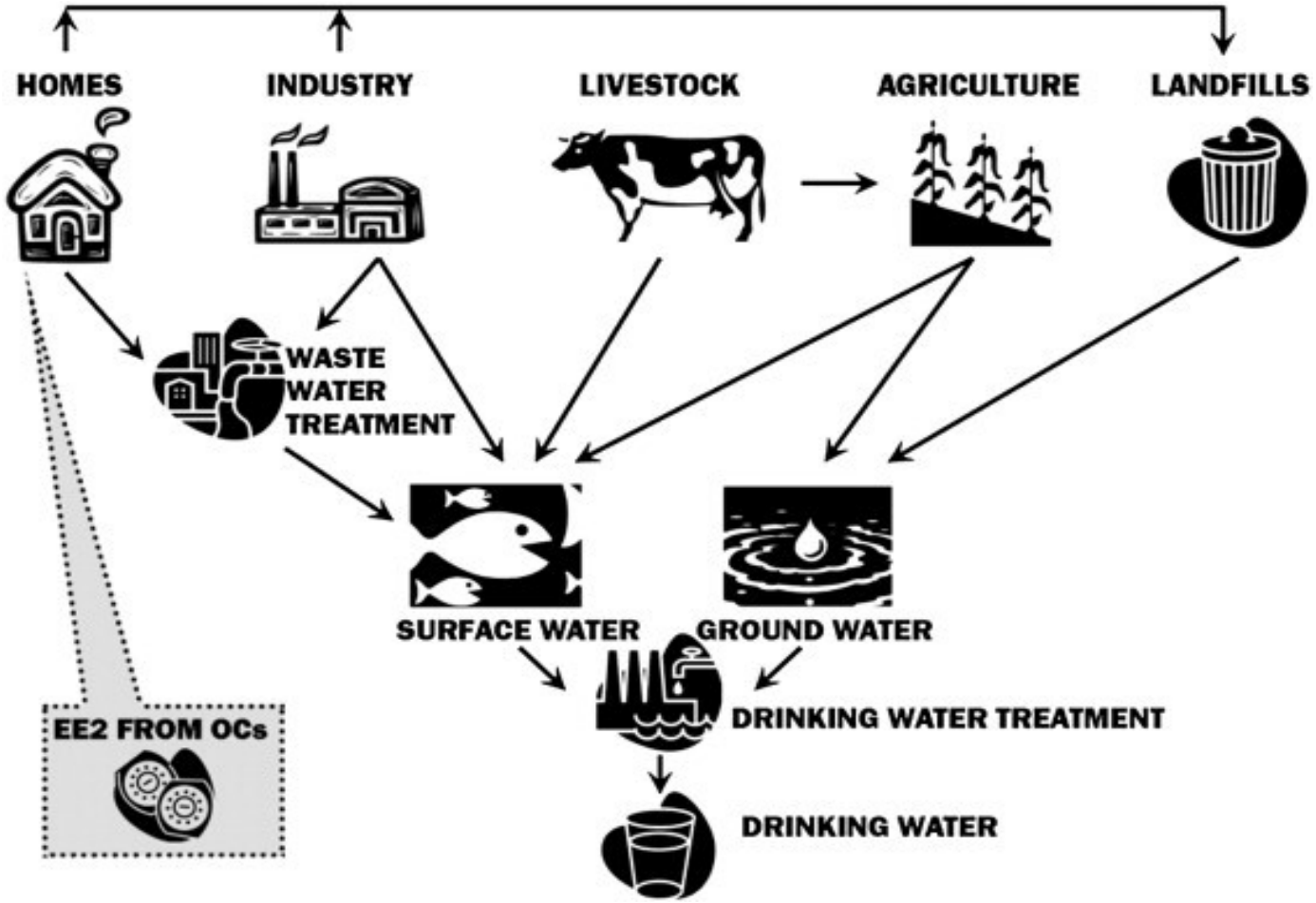
How Do Hormones Get into Drinking Water?



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

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Health Risks of Hormones in Water Supplies

- Hormones in water supplies are typically at very low concentrations (ppb or ppt levels)
- Even extremely diluted concentrations of hormone residues can 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 ongoing in the EU and U.S.
  - 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

- 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 U.S. EPA signed the third Unregulated Contaminant Monitoring Rule (UCMR 3)
  - Requires monitoring for 30 contaminants using U.S. EPA and/or consensus organization analytical methods during 2013–2015.
  - U.S. EPA Method 539 is included in UCMR 3:

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

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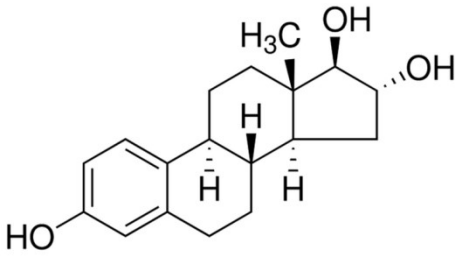
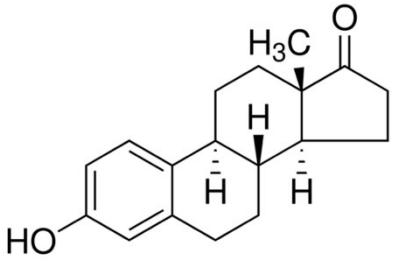
Hormones Monitored: U.S. EPA Method 539

Estrogens

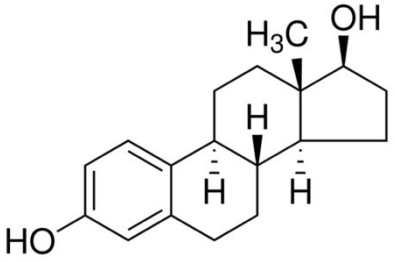
Estriol



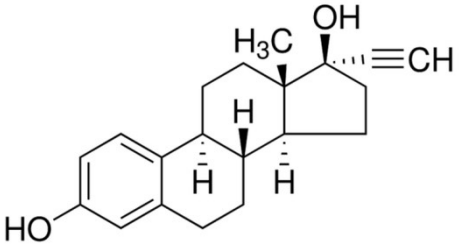
Estrone



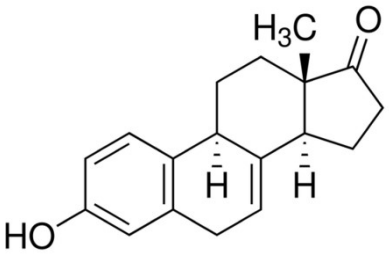
17-β-Estradiol



17-α-Ethynylestradiol

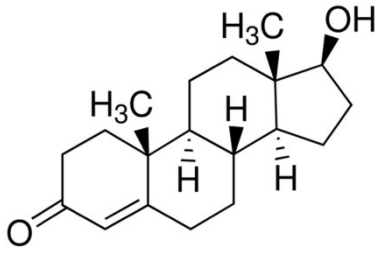


Equilin

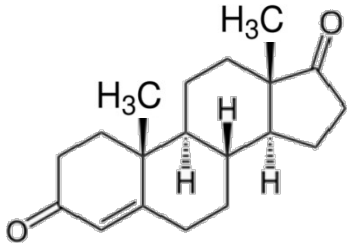


Androgens

Testosterone



Androstenedione



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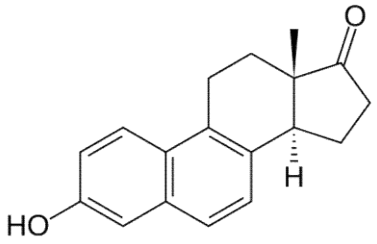
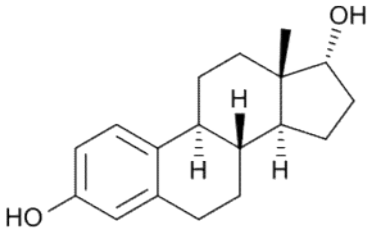
U.S. EPA Method 539.1: Additional Hormones

Estrogens

Equilenin

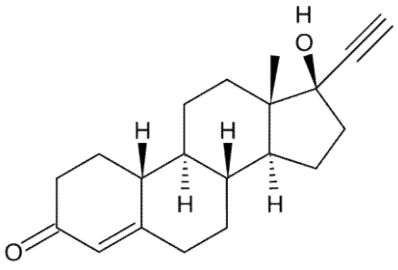


17-α-estradiol

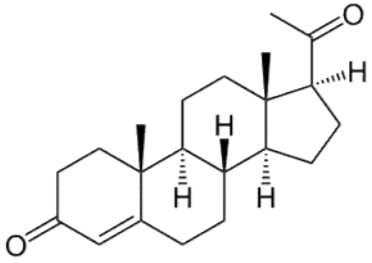


Progestagens

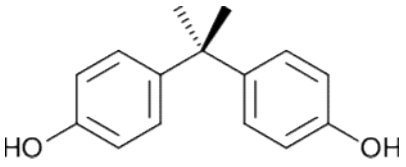
Norethindrone



Progesterone



Bisphenol A



- Use of cartridges specified

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Hormone Analysis: Instrumentation

- 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 Endura™ Triple Quadrupole Mass Spectrometer

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Dionex AutoTrace 280 Solid-Phase Extraction

- 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

SPE Cartridges

- 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

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Sample Concentration: Solid Phase Extraction

0.5 L Water

- Sodium Omadine (Biocide)
- Sodium Chloride (Extraction Salt)
- Ethynylestradiol-d_4 (MS Surrogate)
- + Hormones

Condition	MeOH; Water; N_2
Load	10 mL/min
Rinse	2% Acetic Acid; Water
Dry	N_2, 10 min
Elute	2 x 3 mL + 4 mL MeOH

Concentrate to dryness (N_2, 40 °C) + 1 mL 50% MeOH + MS Internal Standards

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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

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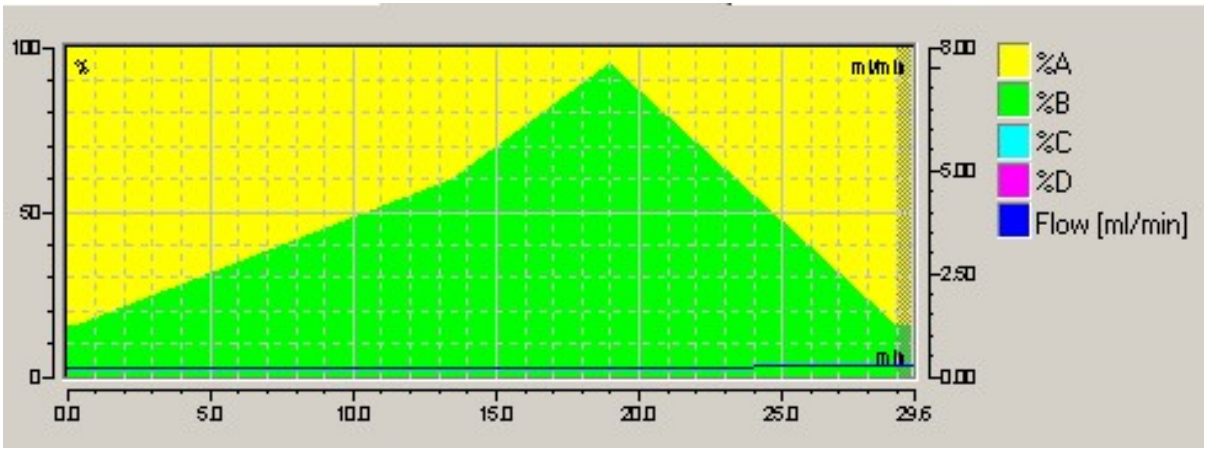
HPLC Conditions

Injection volume	50 µL
Column temperature	30 °C
Mobile phase A	1 mM Ammonium fluoride in water
Mobile phase B	1 mM Ammonium fluoride in methanol
Flow rate	200 µL/min

Gradient:



Time (min)

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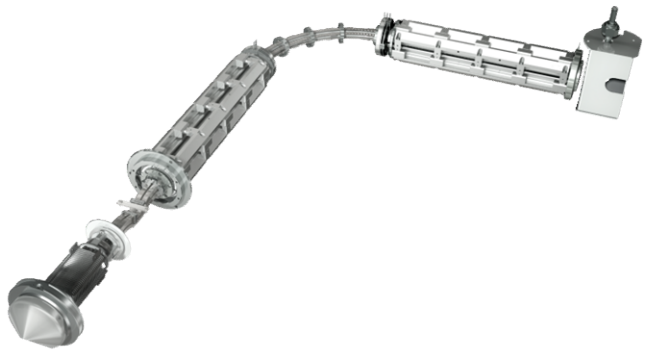
TSQ Endura MS



TSQ Endura MS

Extreme Quantitative Value

- Best-in-class performance
- Unprecedented usability
- Exceptional robustness

AIM
TECHNOLOGY

Active Ion Management:
Precision design of all electric fields, optimized in concert, to produce maximum signal and prevent contamination.

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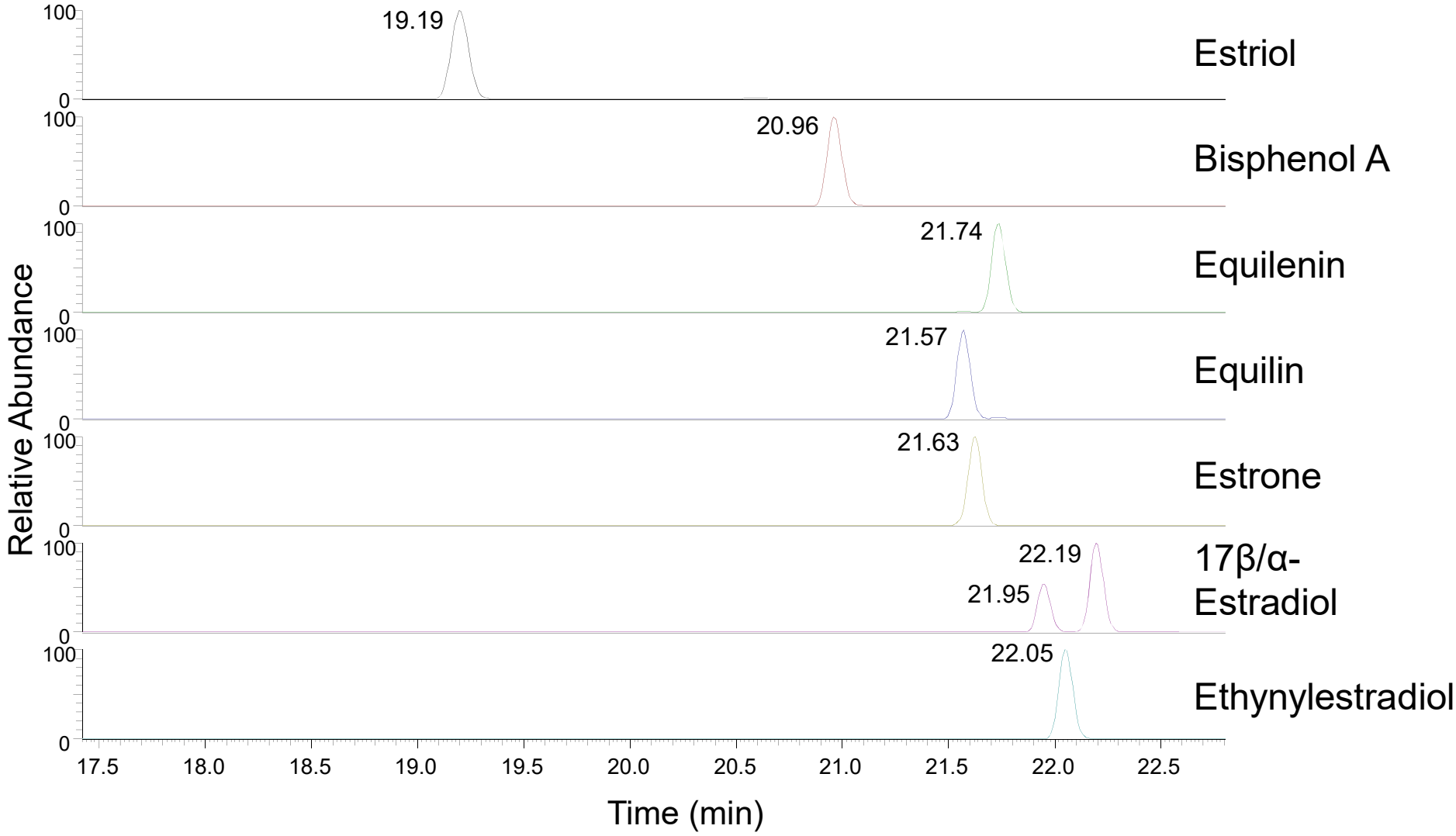
Mass Spectrometry Conditions

Ion source	HESI III
Spray voltage (Polarity Switching)	3000 V (-) 3250 V (+)
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	15 arbitrary units
Sweep gas pressure	1 arbitrary units
Ion transfer capillary temperature	300 °C
Vaporizer temperature	350 °C
Scan type	SRM
Q1 and Q3 peak width (FWHM)	0.7 Da
Collision gas and pressure	Argon at 1.5 mTorr

Analyte Chromatograms – Negative Ionization



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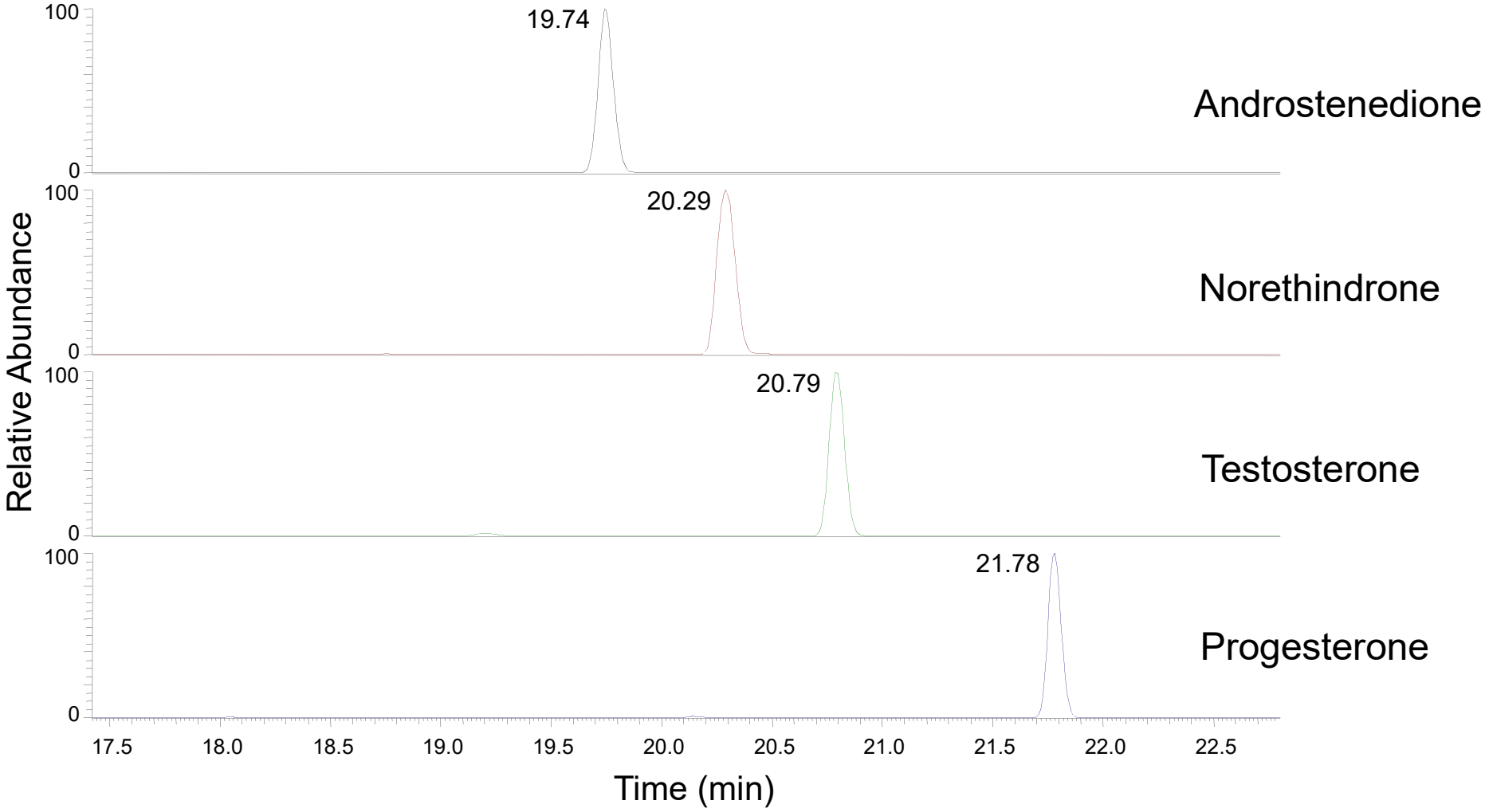
Analyte Chromatograms – Positive Ionization



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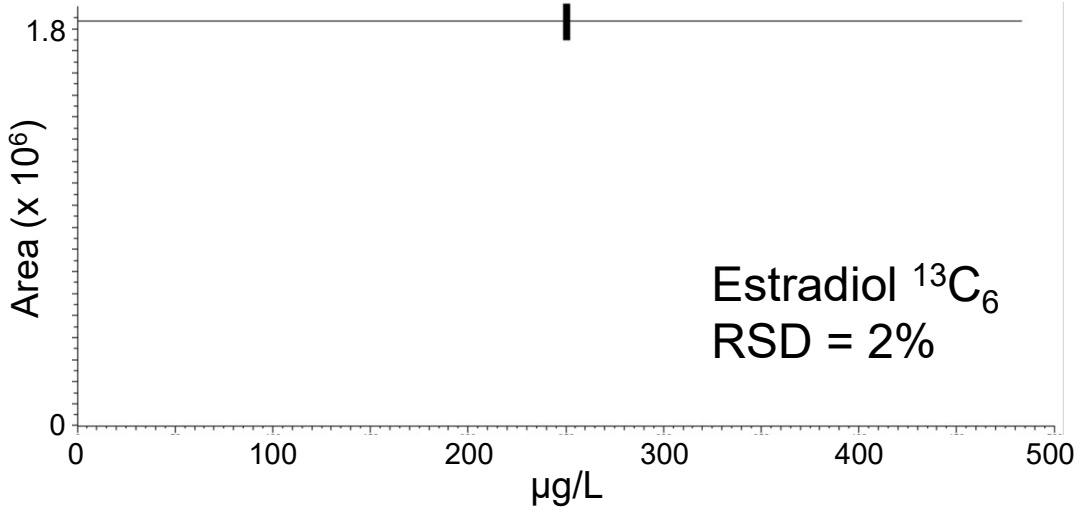
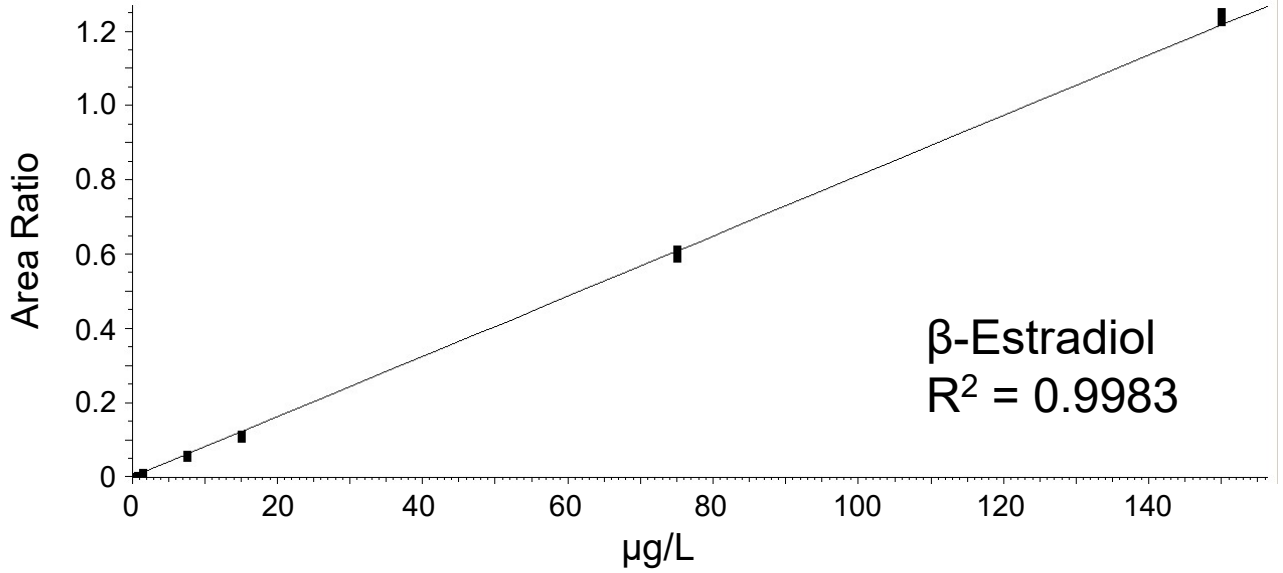
Standard Curve Linearity

- Standard Dilutions
  - Hormones
  - Surrogate
  - Internal Standards





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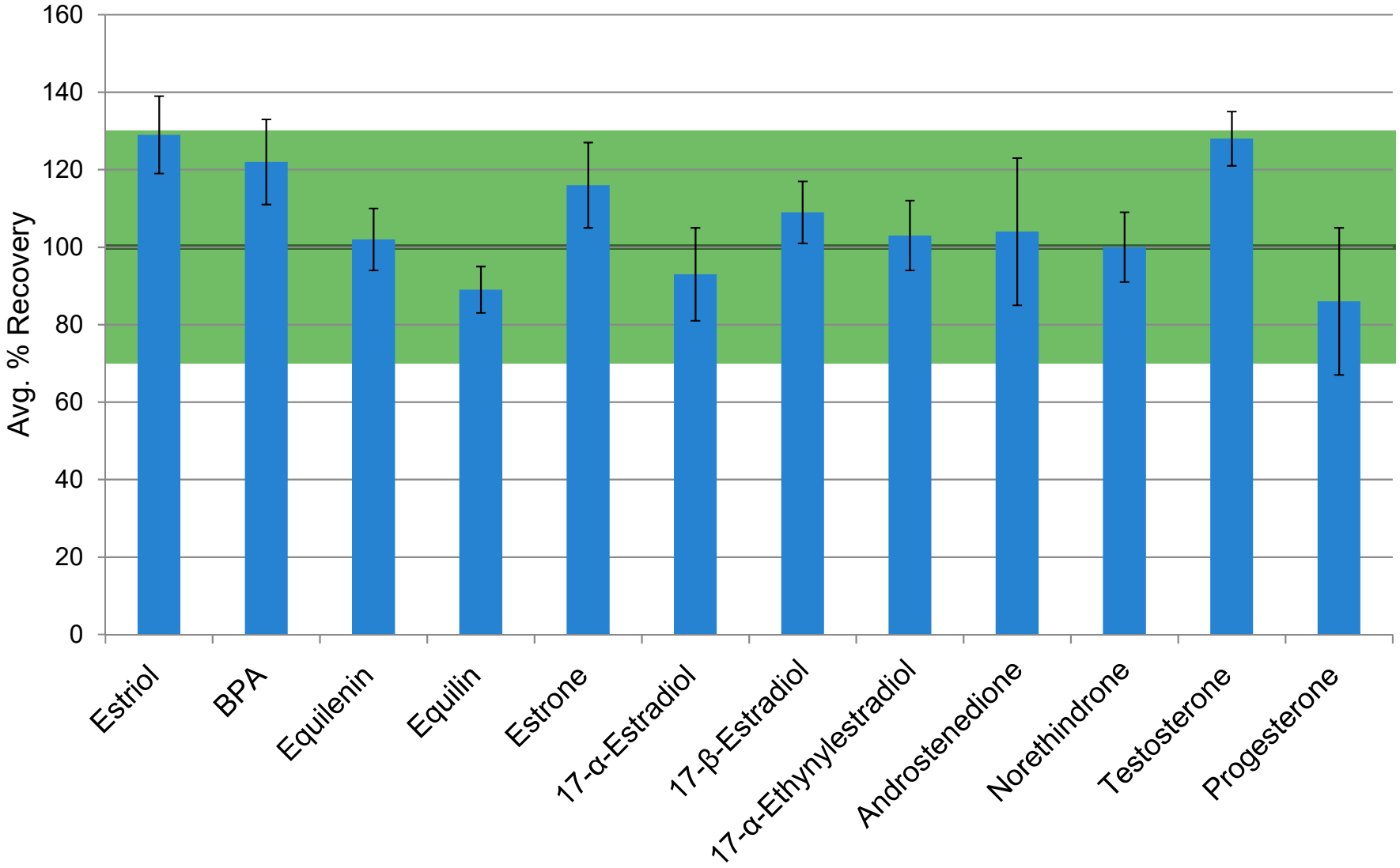
Recovery of Hormones: Fortified Reagent Water



Recovery ranged from 86–129 %

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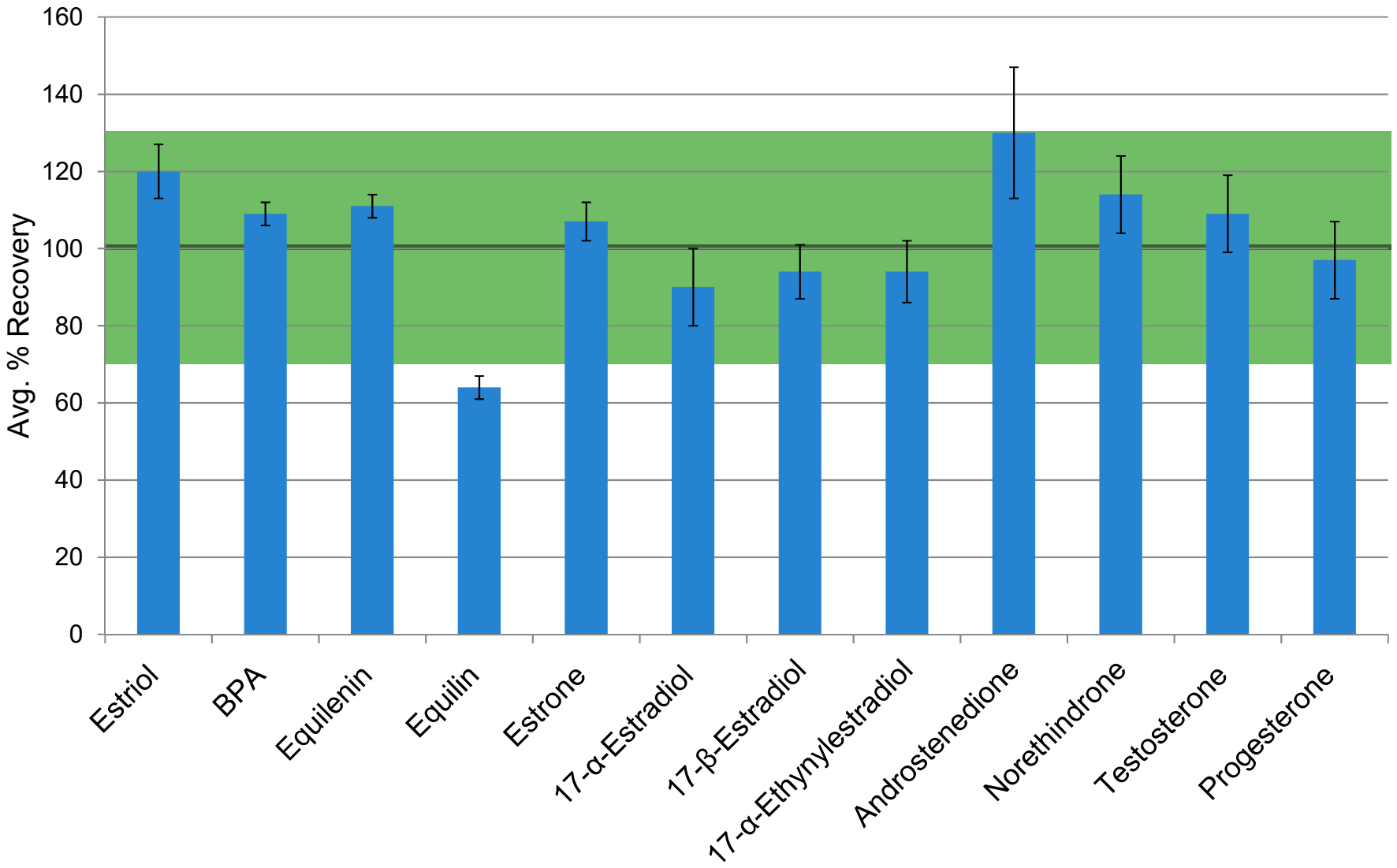
Recovery of Hormones: Fortified Drinking Water



Recovery ranged from 64–130 %

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Lowest Concentration Minimum Reporting Level

Hormone	Draft Method Data (ng/L)	Calculated (ng/L)*
Estriol	4.3	63
Bisphenol A	111	80
Equilenin	2.7	6.7
Equilin	5.2	0.15
Estrone	2.4	3.3
17-α-Estradiol	3.5	1.8
17-β-Estradiol	6	0.67
17-α-Ethynylestradiol	23	9.2
Androstenedione	0.17	0.16
Norethindrone	0.36	0.71
Testosterone	0.031	0.021
Progesterone	0.072	0.069

*Four replicates of seven concentrations

LCMRLs were comparable if not better

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Conclusion

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

Dionex AutoTrace 280 Solid-phase Extraction Instrument

UltiMate 3000 LC System

TSQ Endura MS System

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

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Thermo Scientific Dionex Sample Prep Product Line

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

Dionex AutoTrace 280 Solid-Phase Extraction Instrument

Dionex SolEx SPE Cartridges

Thermo Scientific™ Rocket™ Evaporator System

Thank You!

ThermoFisher SCIENTIFIC

Recovery of Hormones: Fortified Reagent Water

Hormone	Fortified Conc. (µg/L)	Avg. % Recovery	% RSD
Estriol	12	129	10
BPA	100	122	11
Equilenin	5	102	8
Equilin	7.5	89	6
Estrone	5	116	11
17-α-Estradiol	5	93	12
17-β-Estradiol	7.5	109	8
17-α-Ethynylestradiol	37	103	9
Androstenedione	0.25	104	19
Norethindrone	10	100	9
Testosterone	0.1	128	7
Progesterone	0.1	86	19

N=5

Recovery ranged from 86–129 %

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Recovery of Hormones: Fortified Drinking Water

Hormone	Fortified Conc. (µg/L)	Avg. % Recovery	% RSD
Estriol	12	120	7
BPA	100	109	3
Equilenin	5	111	3
Equilin	7.5	64	3
Estrone	5	107	5
17-α-Estradiol	5	90	10
17-β-Estradiol	7.5	94	7
17-α-Ethynylestradiol	37	94	8
Androstenedione	0.25	130	17
Norethindrone	10	114	10
Testosterone	0.1	109	10
Progesterone	0.1	97	10

N=5

Recovery ranged from 64-130 %

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