

ThermoFisher
S C I E N T I F I C

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

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Thermo Fisher Scientific

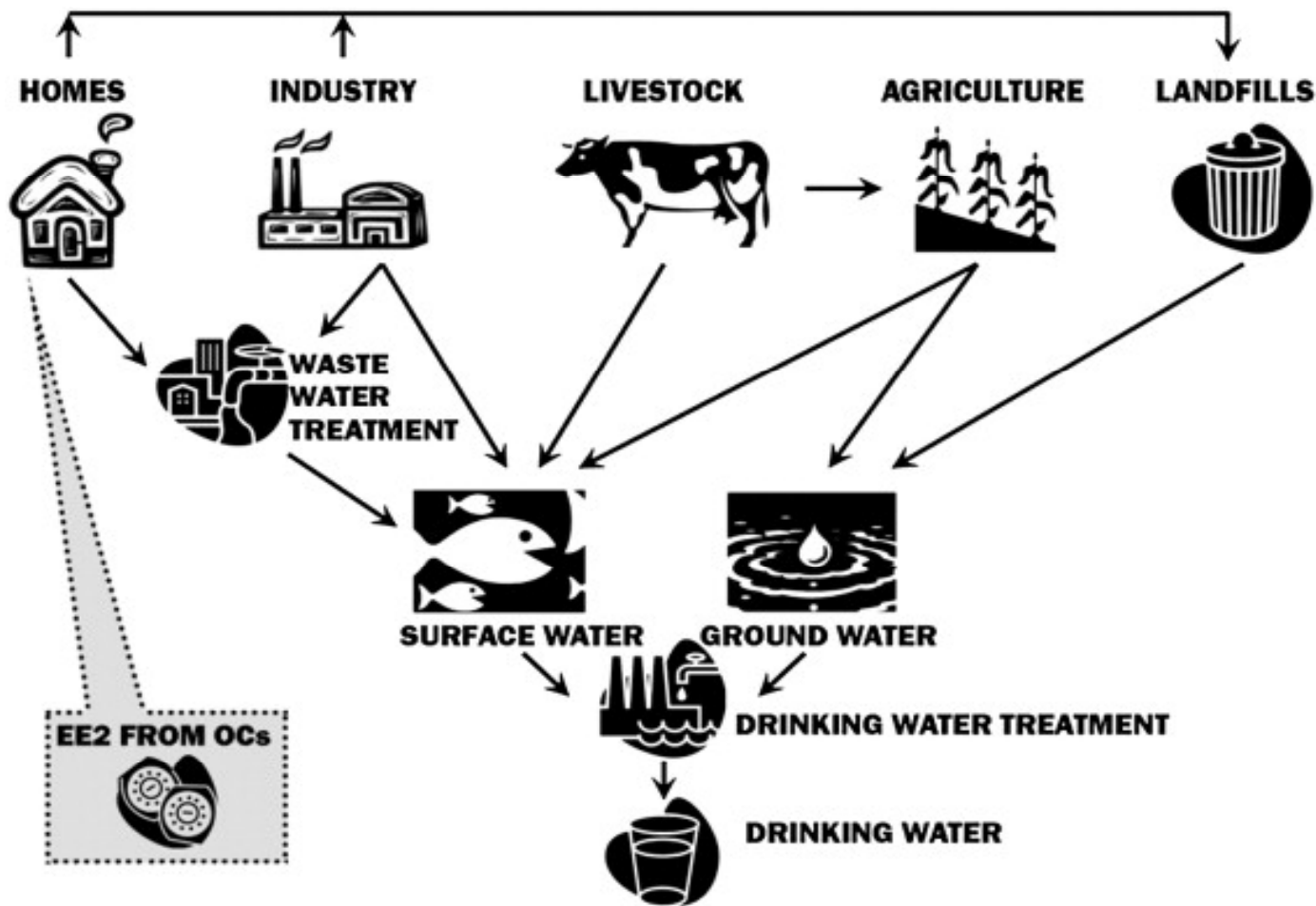
The world leader in serving science

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.
- Other research has 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

- Hormones in water supplies are typically at very low concentrations (ppb or ppt levels)
- Even extremely diluted concentrations of hormone residues can harm aquatic life
- 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
 - 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:

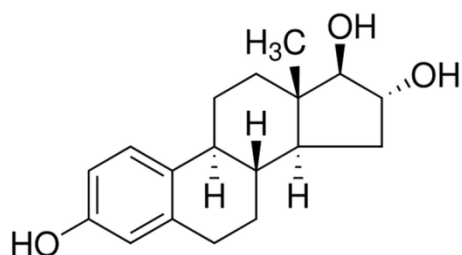
Screening Survey	
7 Hormones using EPA Method 539 (LC/MS/MS):	
17- β -estradiol	estrone.
17- α -ethynylestradiol (ethinyl estradiol)	testosterone.
estriol (16- α -hydroxy-17- β -estradiol)	4-androstene-3,17-dione.
equilin.	



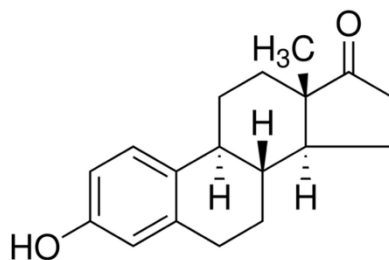
Hormones Monitored: U.S. EPA Method 539

Estrogens

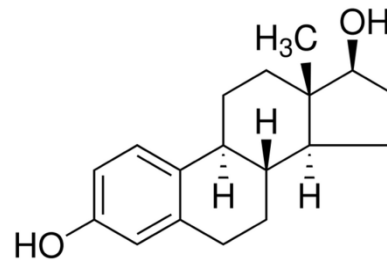
Estriol



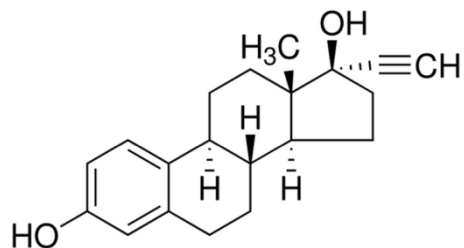
Estrone



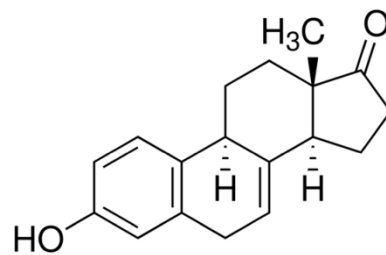
17-β-Estradiol



17-α-Ethynylestradiol

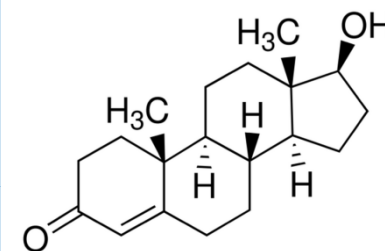


Equilin

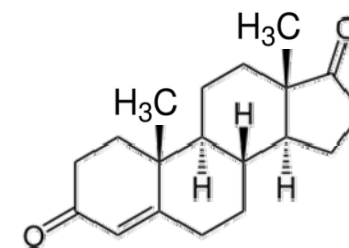


Androgens

Testosterone



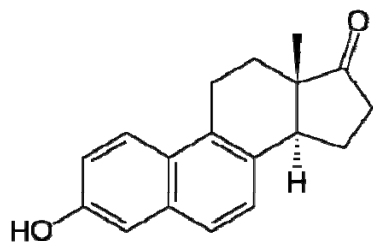
Androstenedione



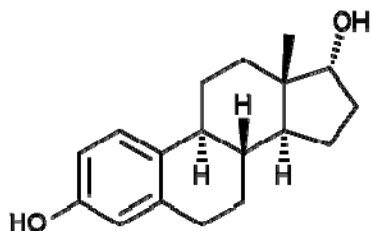
U.S. EPA Method 539.1: Additional Hormones

Estrogens

Equilenin

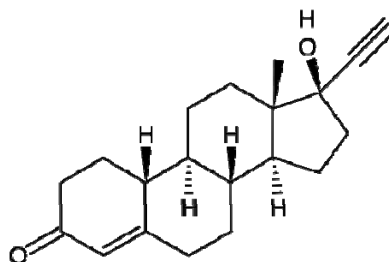


17- α -estradiol

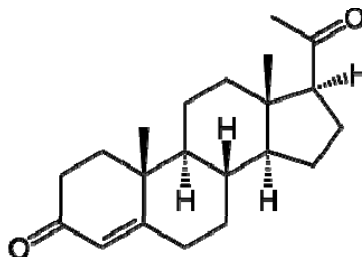


Progestagens

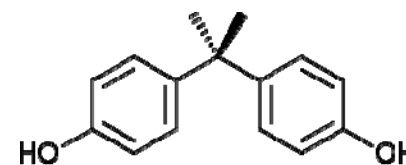
Norethindrone



Progesterone



Bisphenol A



- Use of cartridges specified

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



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

AutoTrace 280 Fluid Schematic

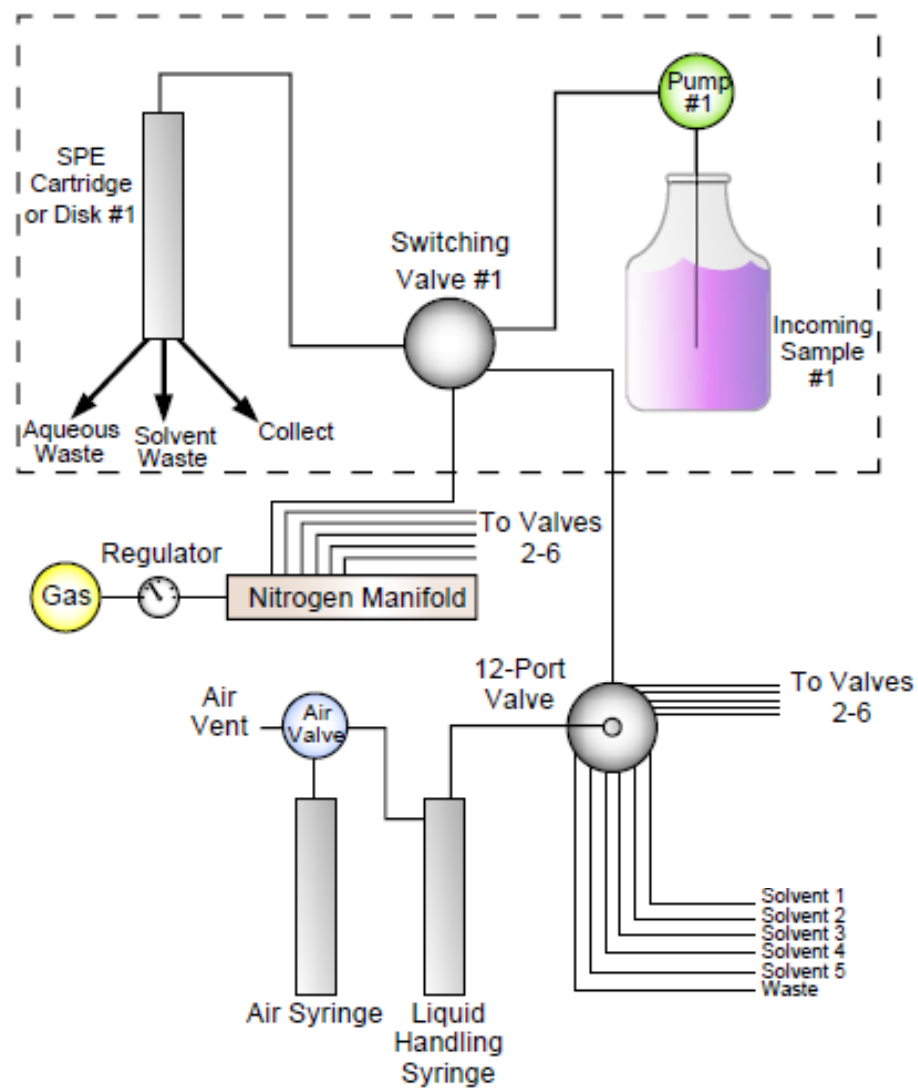


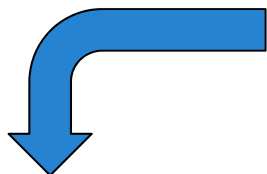
Figure 2-6. AutoTrace 280 Fluid Connections

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



Sample Concentration: Solid Phase Extraction

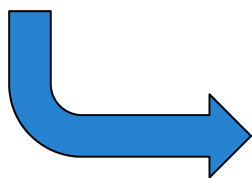


0.5 L
Water

Sodium Omadine (Biocide)
Sodium Chloride (Extraction Salt)
Ethinylestradiol- 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
(N₂, 40 °C)

+

1 mL
50% MeOH

+

MS
Internal
Standards

HPLC System

- UltiMate 3000 RSLC system



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

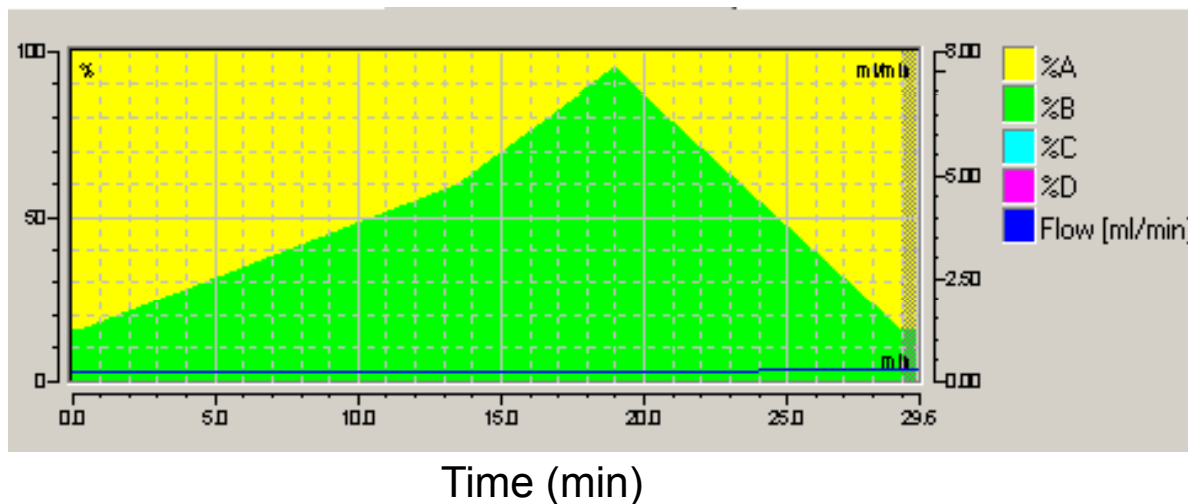


- Thermo Scientific™ Acclaim™
Rapid Separation LC (RSLC)
Polar Advantage II column
- 2.2 μm particle size, 2.1 \times 150 mm
 - pH 1.5–10

HPLC Conditions

Injection volume	50 μ L
Column temperature	30 $^{\circ}$ C
Mobile phase A	1 mM Ammonium fluoride in water
Mobile phase B	1 mM Ammonium fluoride in methanol
Flow rate	200 μ L/min

Gradient:



TSQ Endura MS



TSQ Endura MS

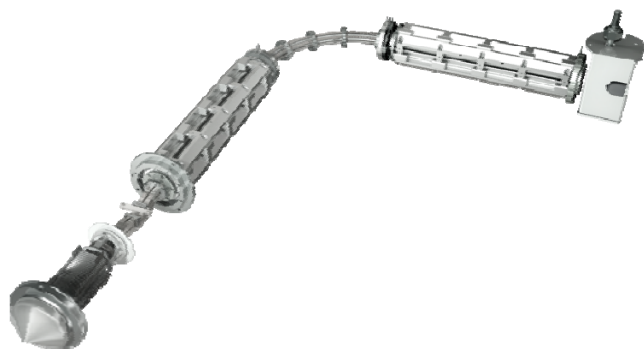
Extreme Quantitative Value

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

AIM TECHNOLOGY

Active Ion Management:

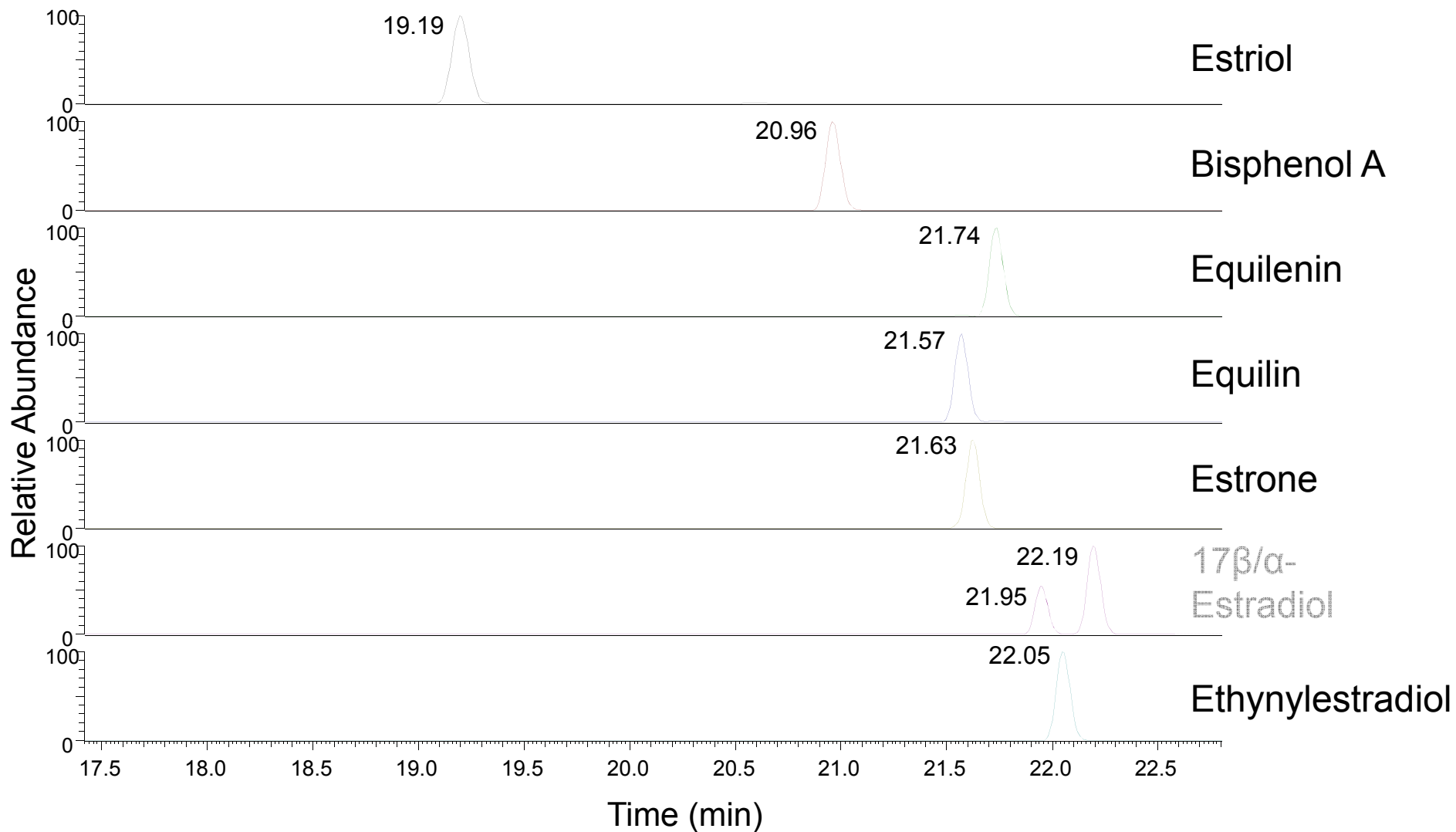
Precision design of all electric fields, optimized in concert, to produce maximum signal and prevent contamination.



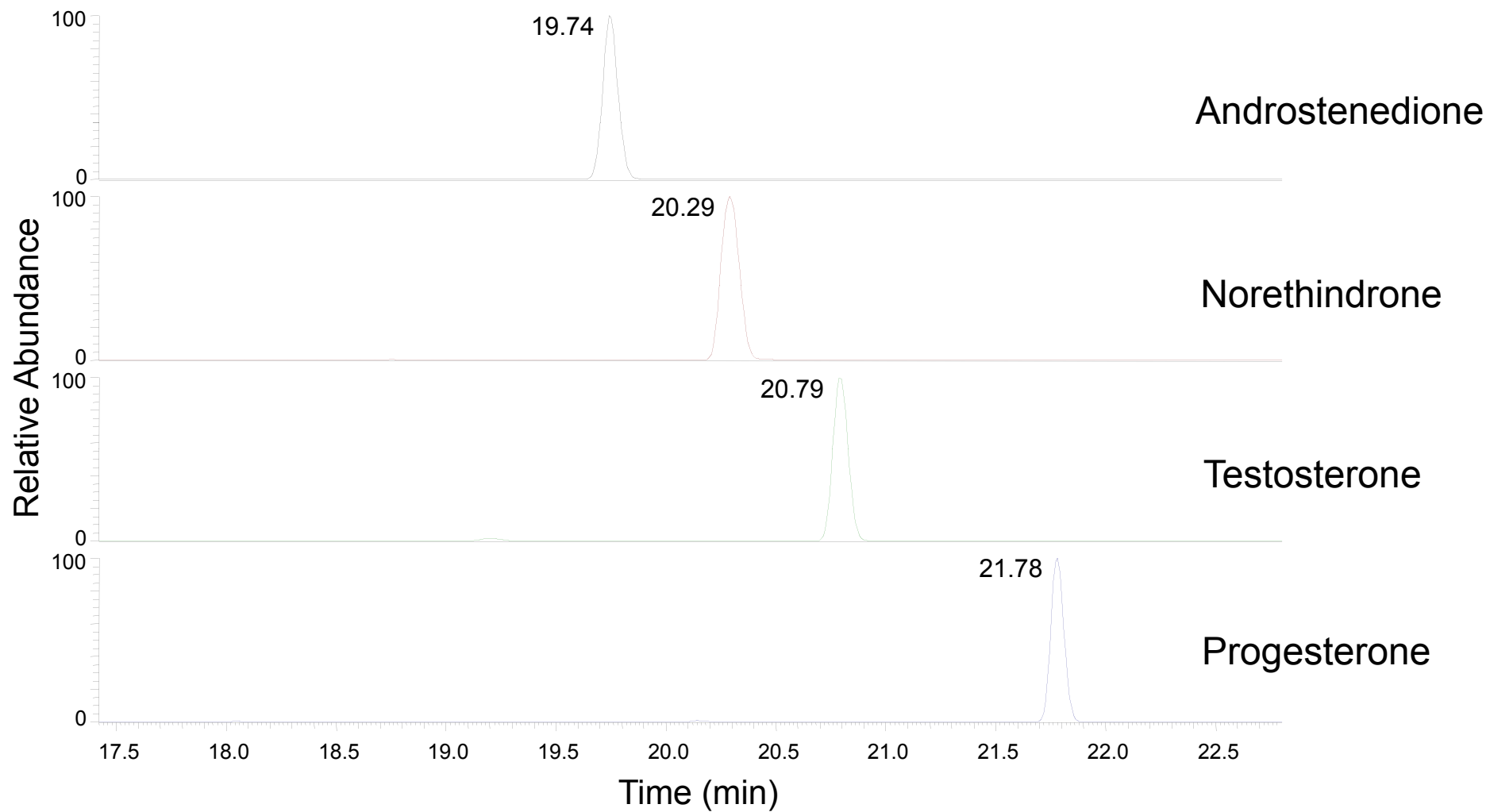
Mass Spectrometry Conditions

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

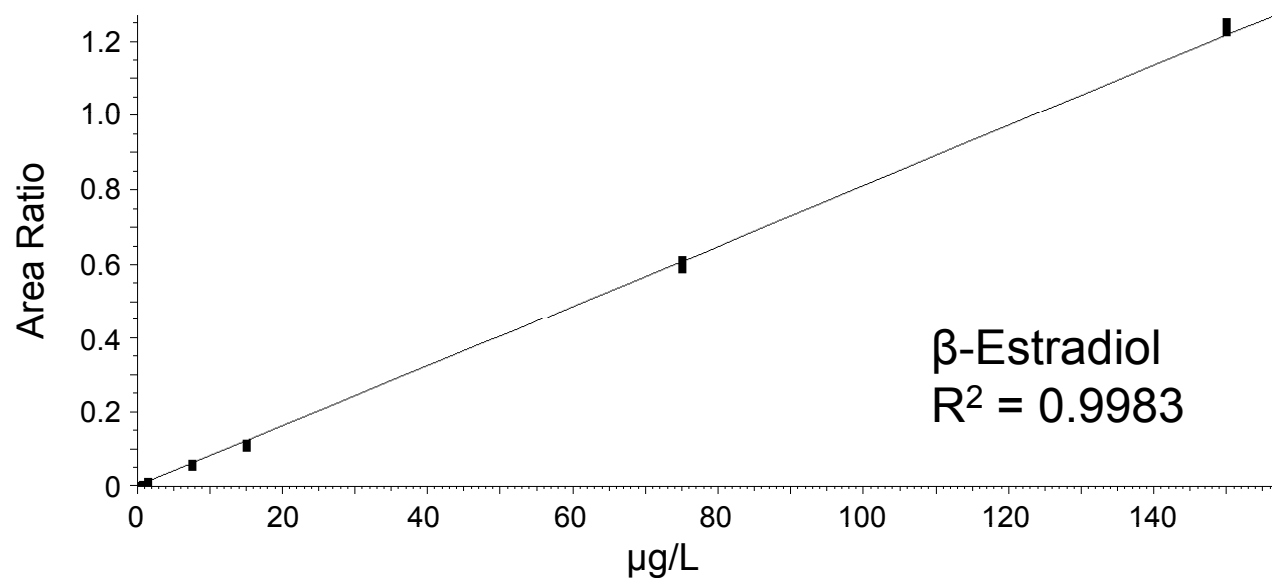
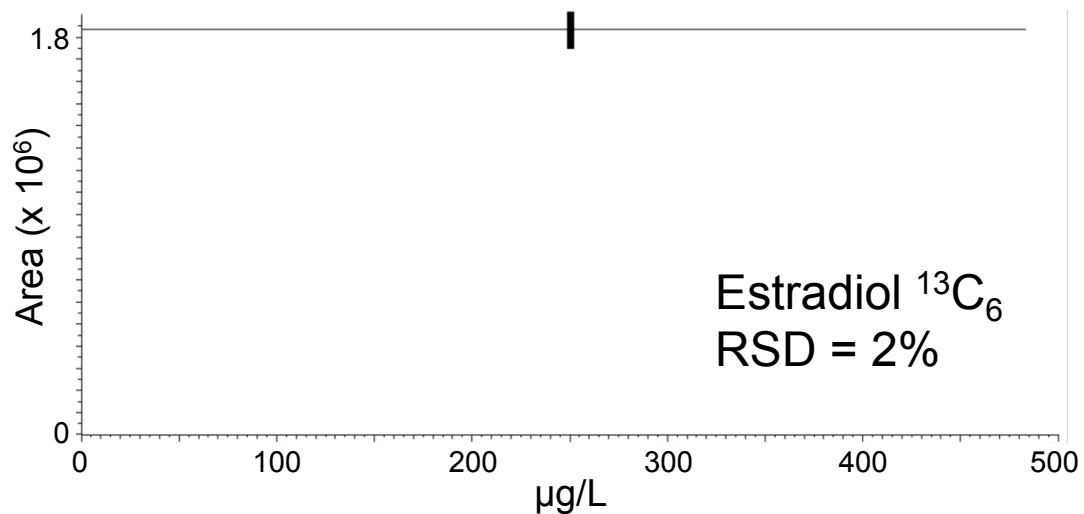


Analyte Chromatograms – Positive Ionization

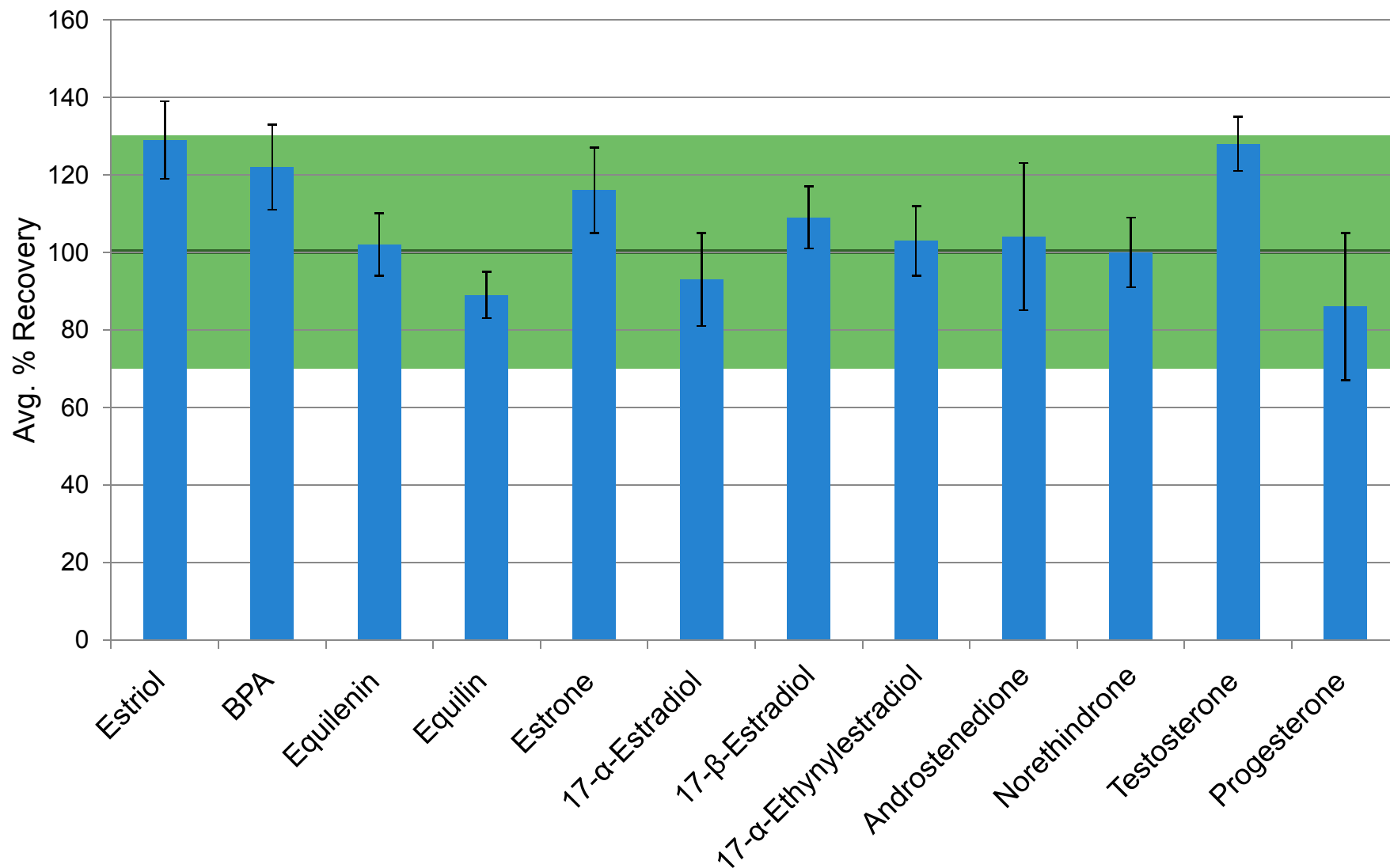


Standard Curve Linearity

- Standard Dilutions
 - Hormones
 - Surrogate
 - Internal Standards

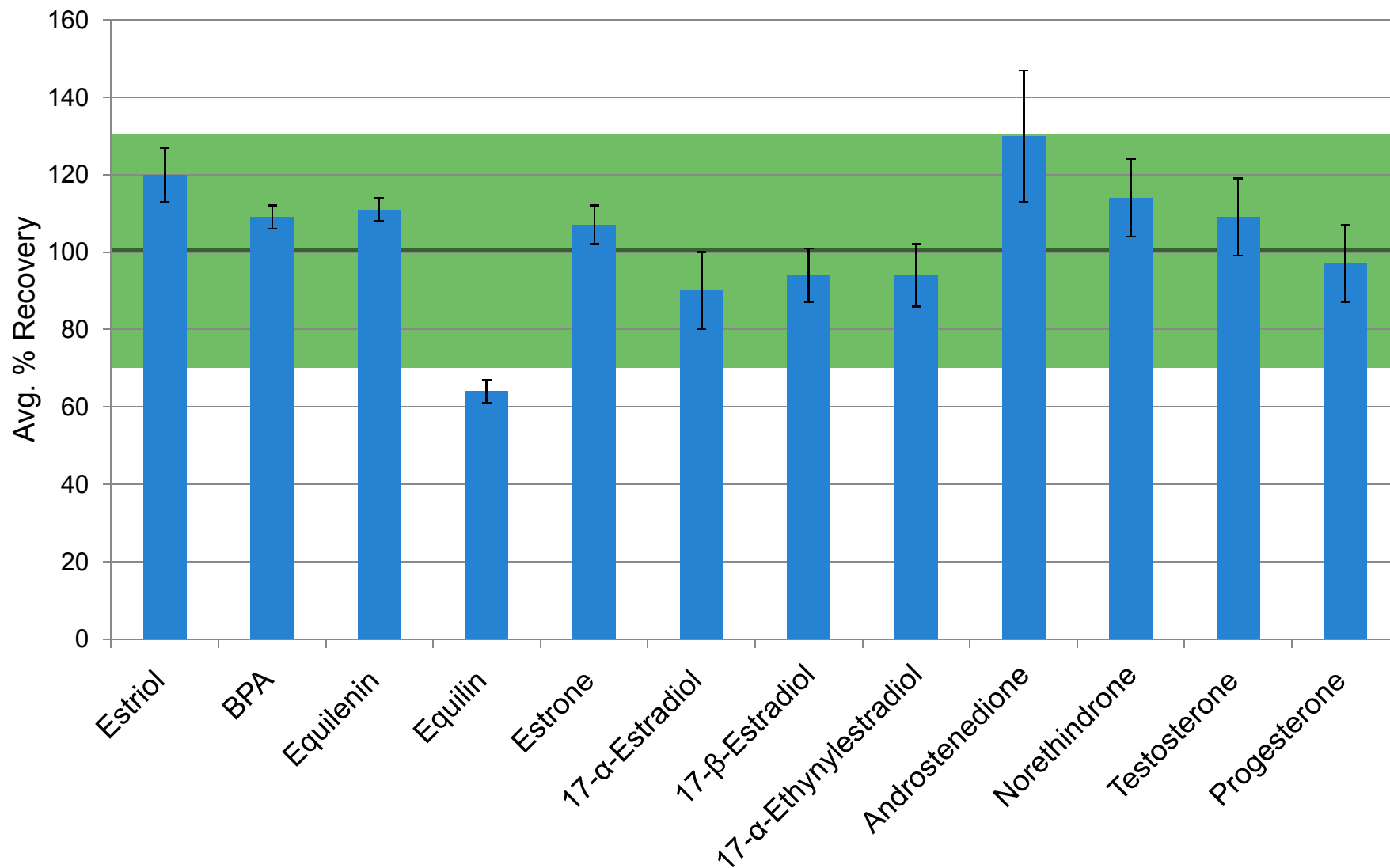


Recovery of Hormones: Fortified Reagent Water



Recovery ranged from 86–129 %

Recovery of Hormones: Fortified Drinking Water



Recovery ranged from 64–130 %

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 -Ethinylestradiol	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

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_w ater concentrated with ~100% recovery
- Low ng/L LCMRLs

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 for your interest!

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 %

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 -Ethinylestradiol	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 %