Challenges in Analyzing 7 Hormones in Drinking Water System Using Automated Extractor and LC/MS/MS

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Overview

- Hormones - from both natural and artificial sources
- Hormones in water - impact the environment and aquatic life and can be harmful to human.
- Unregulated Contaminant Monitoring Rule (UCMR3) EPA Method 539 - monitoring seven hormones in large drinking water systems from 2013-2015.

- **EPA 539 target list:**

Estriol	Estrone
Estradiol	Ethynylestradiol
Equilin	Androstenedione
Testosterone	

- Estriol, Estrone and Estradiol – female estrogen
- Testosterone , Androstenedione – male hormone

EPA 539 – UCMR3
Method Reporting Limit (MRL)

LIMS ID		MRL(ppt)
ESTRIO	Estriol	0.80
bESTRA	Estradiol	0.40
aETEST	Ethynylestradiol	0.90
TESTOR	Testosterone	0.10
ESTRON	Estrone	2.00
ANDRST	Androstenedione	0.30
EQUILN	Equilin	4.00

EPA 539 – Sample Collection, Preservation & Handling

- Wear Nitrile Gloves
- Preservative:
  - Sodium Thiosulfate: 80mg/L -- Removes free Chlorine
  - 2-mercaptopyridine-1-oxide: 65mg/L -- Microbial inhibitor
- A Field Reagent Blank must be handled along with each sample site.
- Store at or below 6^0C and protect from light until analysis.

EPA 539 – Sample Preparation

- Solid Phase Extraction (SPE)
  - HORIZON automated extraction system
  - SPE Disk – 47mm, Octadecyl (C18) sorbent phase
- 1000-mL sample volume
- Concentrate to dryness then reconstitute with 50:50 methanol:water

EPA 539 - Analytical Method

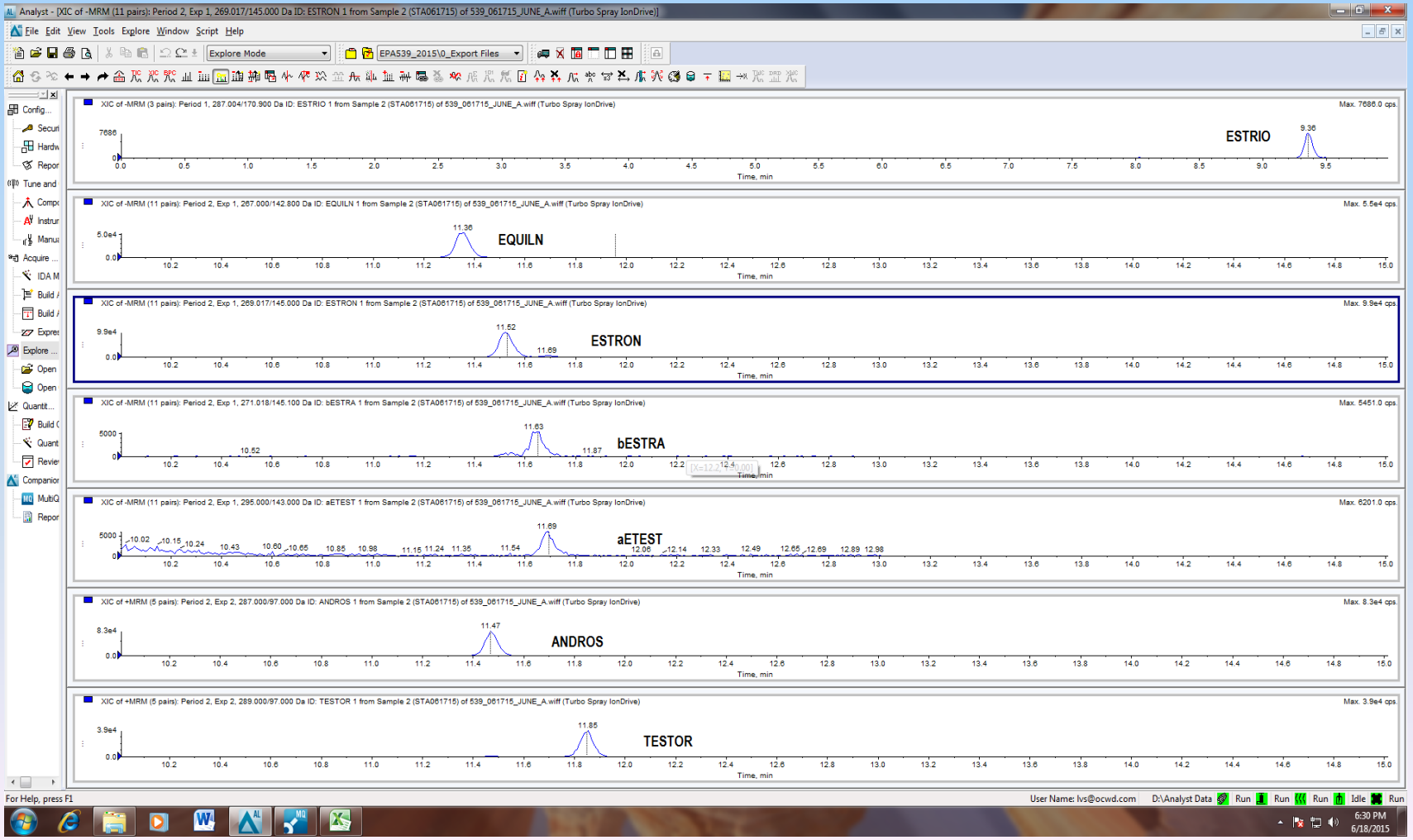
- C18 Column – Phenomenex Kinetex C18 (100 x 2.1mm, 5um)

- Mobile Phases –
  - 0.2% Ammonium Hydroxide in HPLC water
  - 0.2% Ammonium Hydroxide in LCMS grade methanol

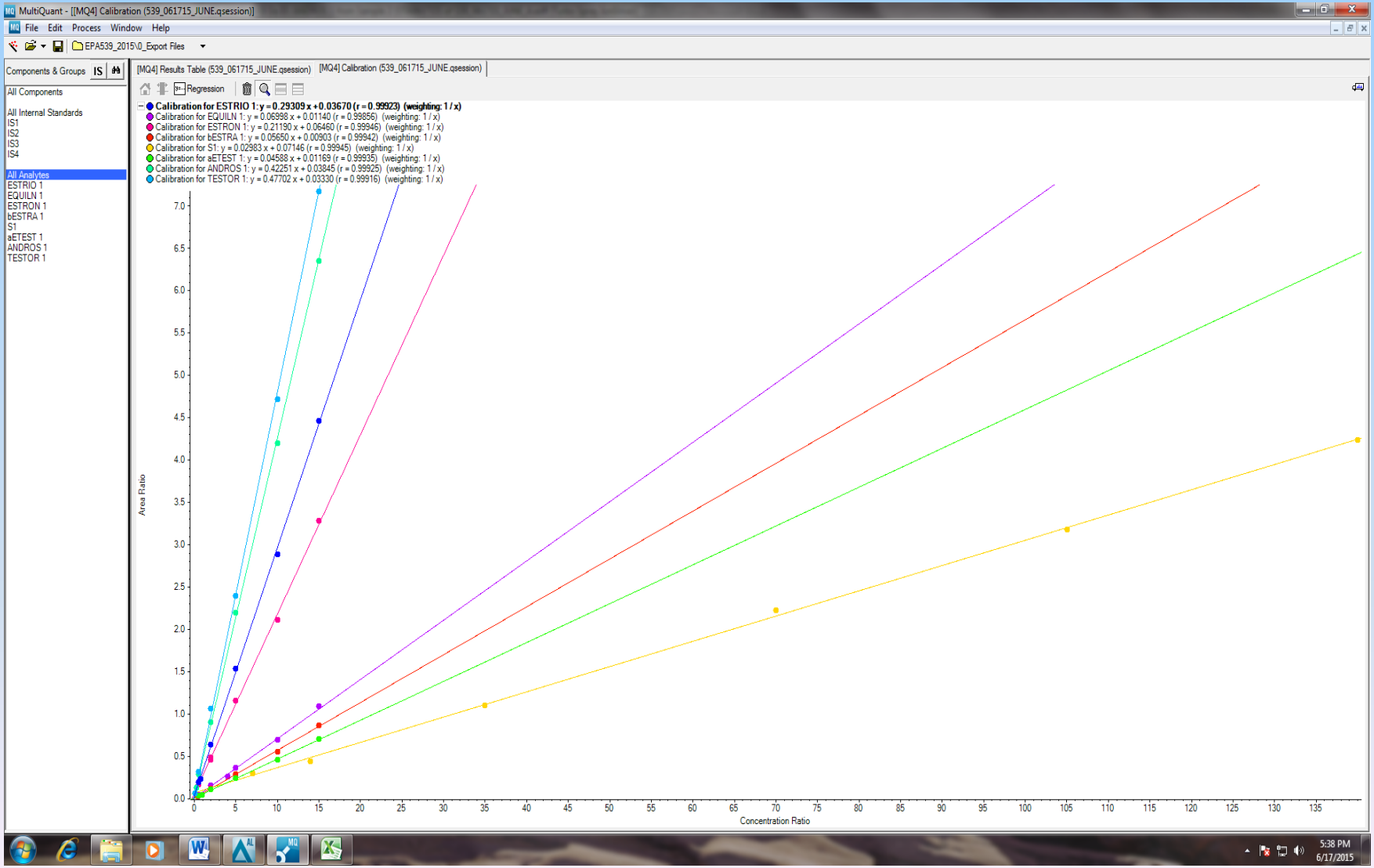
- 10 μL injection volume

EPA 539 - Analytical Method (con't)

- Analysis reduce to 15 min from 50 min (EPA method) to increase productivity



EPA 539 - Calibration Curves



EPA 539
Method Development Challenges

- Low MRL requires by UCMR3 program
- Require MS to analyze in both positive and negative modes and to get maximum sensitivity

EPA 539
Method Development Challenges

Manual Extraction

- Pro
  - Less water in the extract
  - Less time to concentrate the extract to dryness ~ 45min to 1hour
- Con
  - Low % recovery for Quality Control (QC) samples – could not meet UCMR3 requirements
  - Inconsistency in results
  - Re-extract samples increase the cost

EPA 539
Method Development Challenges

Horizon Automated Extractors

- Pro
  - Excellent % recovery for QCs – meet UMCR3 criteria requirement
  - Consistency in results
  - Reduce human errors
- Con
  - Require longer time to concentrate the extract to dryness
    - 2 hour to 3 hours

EPA 539
Method Development Challenges

Stock standards

- Could not get the MRL level custom mix standard at the beginning of the program

- Stock concentrations between two vendors do not match - needs a third vendor to confirm the standard concentration

EPA 539
Method Development Challenges

Stock standards

- Occasionally - same vendor, same concentration but different lot –does not match

- Working standards – needs to make monthly

EPA 539
Method Development Challenges

Extract concentration

- Require time to optimize the temperature and pressure setting of the Turbo Vap Concentrator to achieve the acceptable % recovery

- Problematic compounds - Estriol, Ethynylestradiol – most of the time their % recoveries are below 85%

EPA 539
Method Development Challenges

Extract concentration

- Require from 2.0 to 3.0 hours to concentrate the extract to dryness

- Cross contamination in the reagent blank that greater than 1/3 of MRL concentration cause re-extraction of the whole batch of samples

EPA 539
Method/Instrument Validation

- Took 6 months to bring EPA 539 online
- Performed and passed Initial Demonstration of Capability (IDC) - validate analytical method and LC/MS/MS instrument.
- Participated and passed UCMR3 Proficiency Test Sample.

EPA 539
UCMR3 Samples Analysis 2013-2015

- Has been analyzed UCMR3 samples since January 2013 – close to 200 samples
- Ground water – twice in a 12-month consecutive period – 2nd sample is 5 to 7 months from date of first samples
- Samples Results - Non Detect (ND) for all 7 hormone compounds

EPA 539
UCMR3 Samples 2013-2015

Continuing challenges

Occasionally

- Laboratory Fortified Blank (LFB) has low % recovery, failed QCs – Re-extract the whole batch of samples
- Samples matrix cause % recovery of Internal Standard (IS) “Testosterone-d3” failed below 50% recovery acceptance limit
- Request for Re-sample to confirm the sample matrix