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The analysis of natural and synthetic estrogens at sub ppt levels in surface water, crude influent and final effluent waters

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Waters European Headquarters

Content

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- Background Information
- Analytical Considerations
- Sample Preparation
- Separation and Detection
- Summary

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Natural and Synthetic Estrogens Some Background

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- Estrogens are routinely used either as contraceptive medicines or in hormone replacement therapy and can enter aquatic environments via the discharge of final effluent waters.

- Compounds can include:

  - 17 alpha ethinyl estradiol (oral contraceptive and HRT)
  - 17 beta estradiol (postmenopausal drug)
  - Estrone (perimenopausal and postmenopausal drug)

- Estrogens are believed to have a negative effect on aquatic environments by disrupting the hormonal systems of fish, which is thought to cause demasculation of aquatic animals.

EU Water Framework Directive
Overview

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24.8.2013 EN Official Journal of the European Union L 226/1

I

(Legislative acts)

DIRECTIVES

DIRECTIVE 2013/39/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 12 August 2013

amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy

(Text with EEA relevance)

EU Water Framework Directive
Overview

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- Addition of 12 new substances to the list of 33:
  - Plant protection product substances: aclonifen, bifenox, cypermethrin, dicofol*, heptachlor*, quinoxyfen*
  - Substances used in biocide products: cybutryne, dichlorvos, terbutryn
  - Industrial Chemicals: PFOS*, hexabromocyclododecane* (HBCDD)
  - Combustion by-products: dioxin* and dioxin-like PCB's*
- Watch list
  - Pharmaceuticals: 17 beta-estradiol, 17 alpha-ethinyl estradiol, diclofenac
- Development of a specific strategy for pharmaceuticals
- It lowers the EQS values of certain substances:
  - Brominated diphenyl ethers, fluoranthene, nickel and PAH's

*priority hazardous substances

EU Water Framework Directive
Watch List

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Article 8b

Watch list

1. The Commission shall establish a watch list of substances for which Union-wide monitoring data are to be gathered for the purpose of supporting future prioritisation exercises in accordance with Article 16(2) of Directive 2000/60/EC, to complement data from, inter alia, analyses and reviews under Article 5 and monitoring programmes under Article 8 of that Directive.

The first watch list shall contain a maximum of 10 substances or groups of substances and shall indicate the monitoring matrices and the possible methods of analysis not entailing excessive costs for each substance. Subject to the availability of methods of analysis not entailing excessive costs, the maximum number of substances or groups of substances that the Commission is allowed to include in the list shall increase by one at each update of the list in accordance with paragraph 2 of this Article, up to a maximum number of 14. The substances to be included in the watch list shall be selected from amongst those for which the information available indicates that they may pose a significant risk at Union level to, or via, the aquatic environment and for which monitoring data are insufficient.

Diclofenac (CAS 15307-79-6), 17-beta-estradiol (E2) (CAS 50-28-2) and 17-alpha-ethinylestradiol (EE2) (CAS 57-63-6) shall be included in the first watch list, in order to gather monitoring data for the purpose of facilitating the determination of appropriate measures to address the risk posed by those substances.

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Analytical Challenges in Environmental Testing and Method Development

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- **Sensitivity** is required to accurately identify contaminants in a wide variety sample matrices
  - Regulated methods
  - Emerging contaminants
- High **throughput** is a necessity
  - Hundreds of samples
  - Fast turnaround time
- Method **ruggedness** and **reliability** is essential
  - Co-eluting endogenous materials can result in reduced assay accuracy
- **Data quality** must be maintained
  - Better, more informed decisions

Method Requirements:
Multi-Class vs. Compound-specific analysis

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	Multi-Residue / Multi-Class	Compound or Class Specific
Entire procedure (sample prep & analytical method)	Cover a diverse set of analytes	Specific for one compound or class of compounds
Sample Preparation Protocol	Minimum Steps	Multi-step
Goal of Sample Cleanup	Speed Recovery & cleanup are compromised for a large number of analytes	Maximizing recovery & matrix cleanup Minimizing interference/ion suppression
Level of Sample Cleanup	Minimum/moderate	Maximum
Detection Techniques	Tandem MS, Time-of-Flight	Tandem MS, Single quad MS, UV, FLR, ELS, GC* (FID or MS)

**GC typically requires a higher level of sample cleanup*

Structures and Physical Properties

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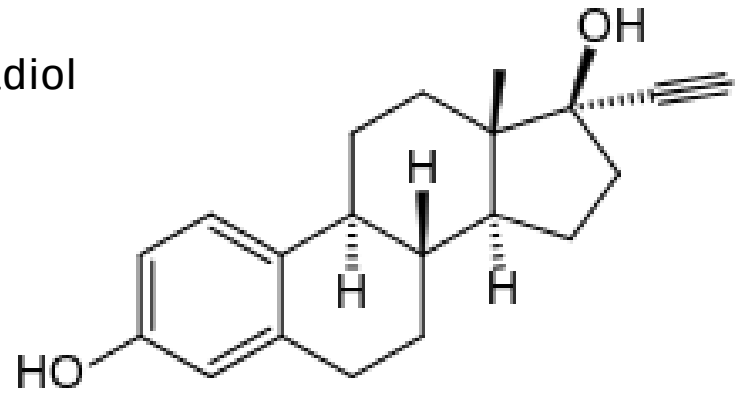
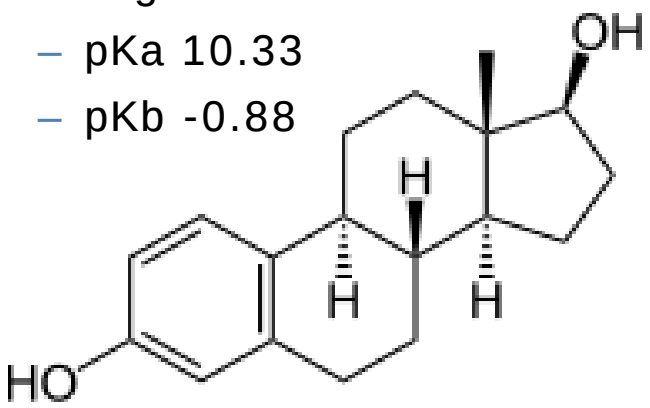
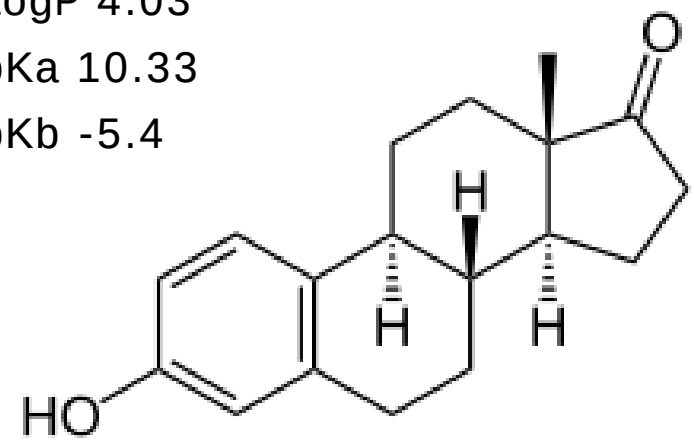
- 17α Ethinyl Estradiol
  - LogP 3.67
  - pKa 10.33
  - pKb -1.7



- 17β Estradiol
  - LogP 3.57
  - pKa 10.33
  - pKb -0.88



- Estrone
  - LogP 4.03
  - pKa 10.33
  - pKb -5.4



Challenges of Steroid Hormone Analysis

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- Very low limits of detection required
  - Surface waters MAC
    - 17α ethinyl estradiol 0.000035ug/l
    - 17β estradiol 0.0004ug/l
- Compounds do not ionise well in ESI or APCI
- Ion Suppression from matrix and extraction process
  - Significant sample clean-up and concentration required
  - Chromatographic separation crucial
- Method has to be robust to allow analysis of both surface and effluent waters

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Good Sample Preparation Provides the Ability to…

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- **Simplify the chromatographic separation**
  - Removing matrix and co-eluting species enables better, more consistent quantitation
- **Reduce analytical variability**
  - Higher, more consistent recovery
  - Minimize matrix effects
  - Less rework
- **Increase column lifetime**
  - Fewer columns need to be replaced
- **Reduce system downtime**
  - Less time spent with wrenches or waiting for service

SPE Sorbents for Environmental Analysis

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- Normal-Phase Sorbents
  - Silica, Alumina, Florisil®, Aminopropyl silica, PSA, Diol silica
- Reversed-Phase Sorbents
  - Oasis® HLB, Oasis PRiME HLB
  - C18, C8 (alkyl bonded silica)
  - Graphitized carbon and activated carbon
- Mixed Mode (ion-exchange/reversed-phase)
  - Oasis® MAX, Oasis® WAX (strong and weak anion-exchange)
  - Oasis® MCX, Oasis® WCX (strong and weak cation-exchange)

Load, Wash and Elute Reversed Phase SPE Method

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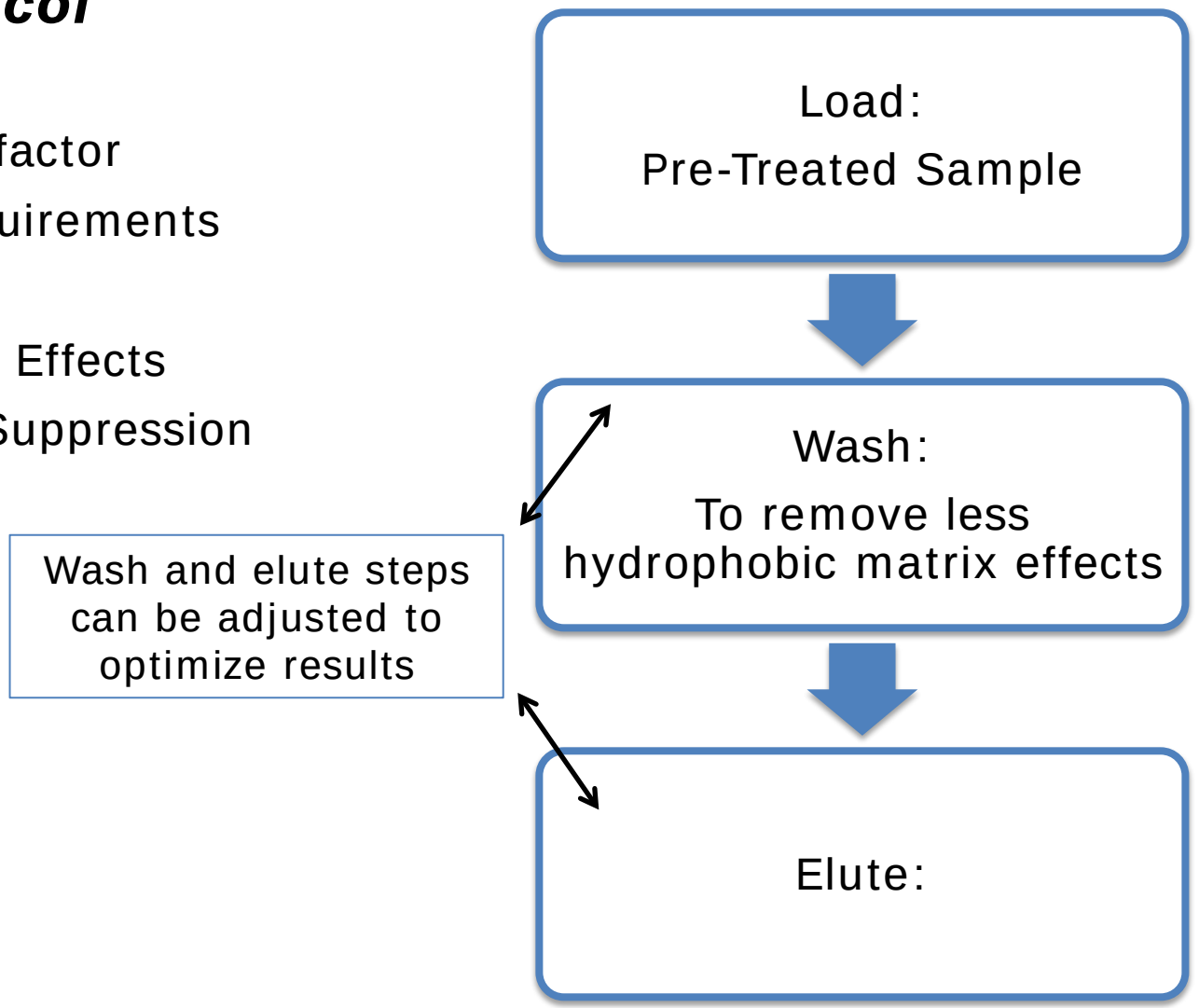
- ***Aim of Protocol***
  - Concentration factor
    - Meet s/n requirements
  - Remove Matrix Effects
    - Reduce Ion Suppression



Sample Preparation for Estrogens in Water

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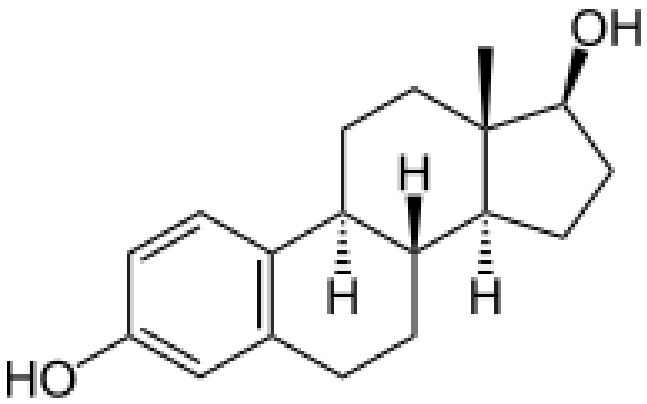
- **Characterize the analytes**
  - They are weak acids pKa >10
  - LogP 3.57 – 4.03 (hydrophobic)
  - -OH groups facilitates normal phase SPE
- **Select the appropriate sorbent**
  - Oasis HLB
  - Oasis MAX (strong anion exchange and HLB)
  - Sep-Pak Silica or alumina



Oasis HLB
A Water-Wettable sorbent

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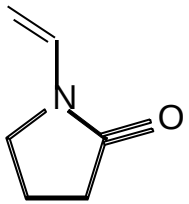
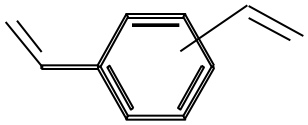
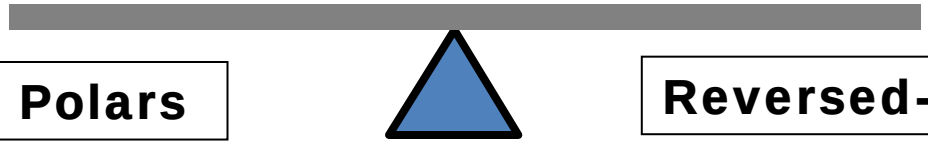
Hydrophilic-Lipophilic Balanced Copolymer



Hydrophilic monomer



Lipophilic monomer



Retention of Polars

Reversed-phase Retention

Oasis HLB is the backbone of all Oasis sorbents

- Stable across pH 1-14
- High recoveries for acids, bases and neutrals
- Water-wettability allows the elimination of condition and equilibration steps
- Will NOT dry out under vacuum or positive pressure, once wetted

Sample Preparation for Estrogens in Surface Water – Oasis HLB

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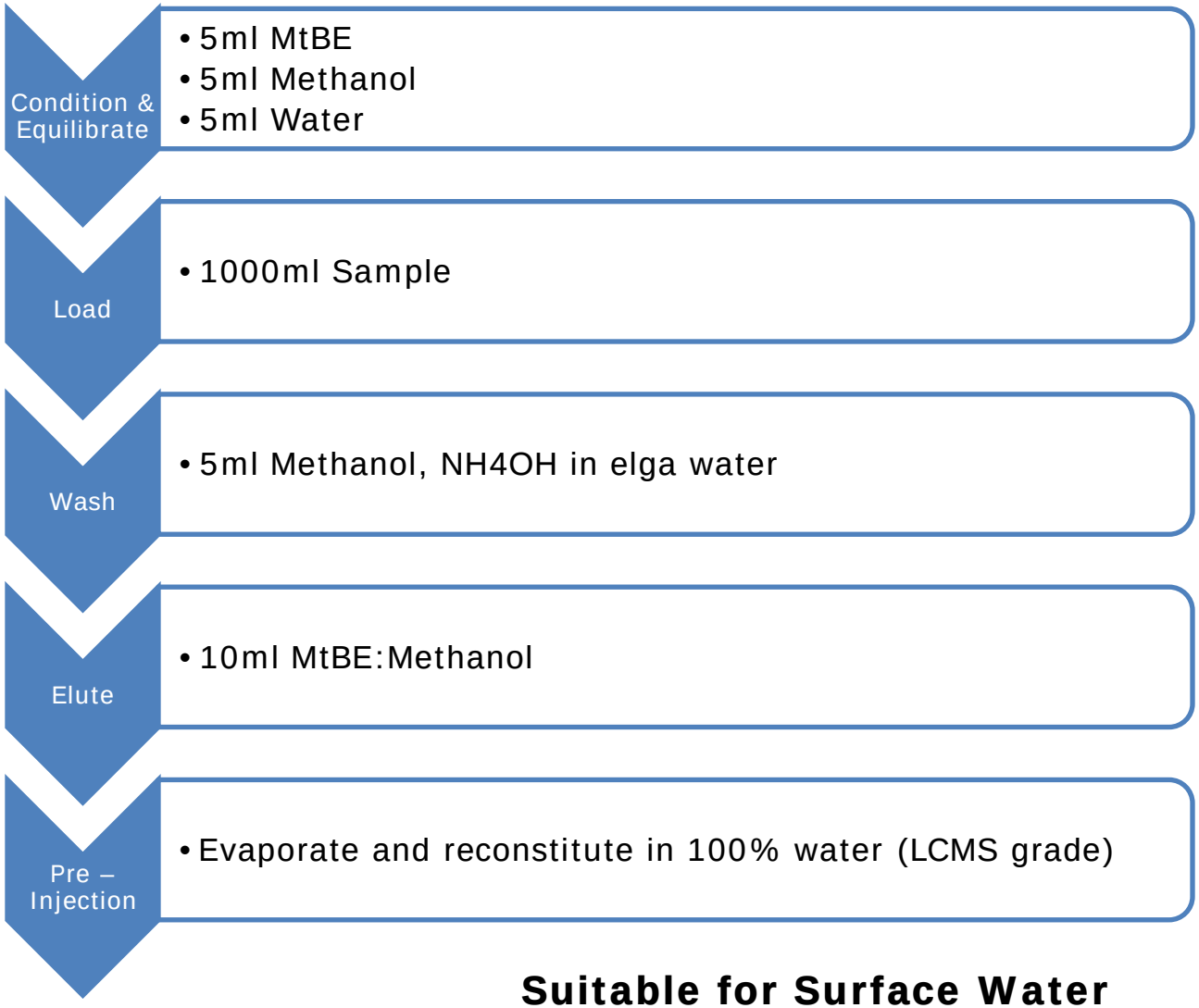

Removes polar compounds and Humic Acid

Suitable for Surface Water

Sample Preparation for estrogens in Effluent Water – Oasis HLB and Sep-Pak Silica

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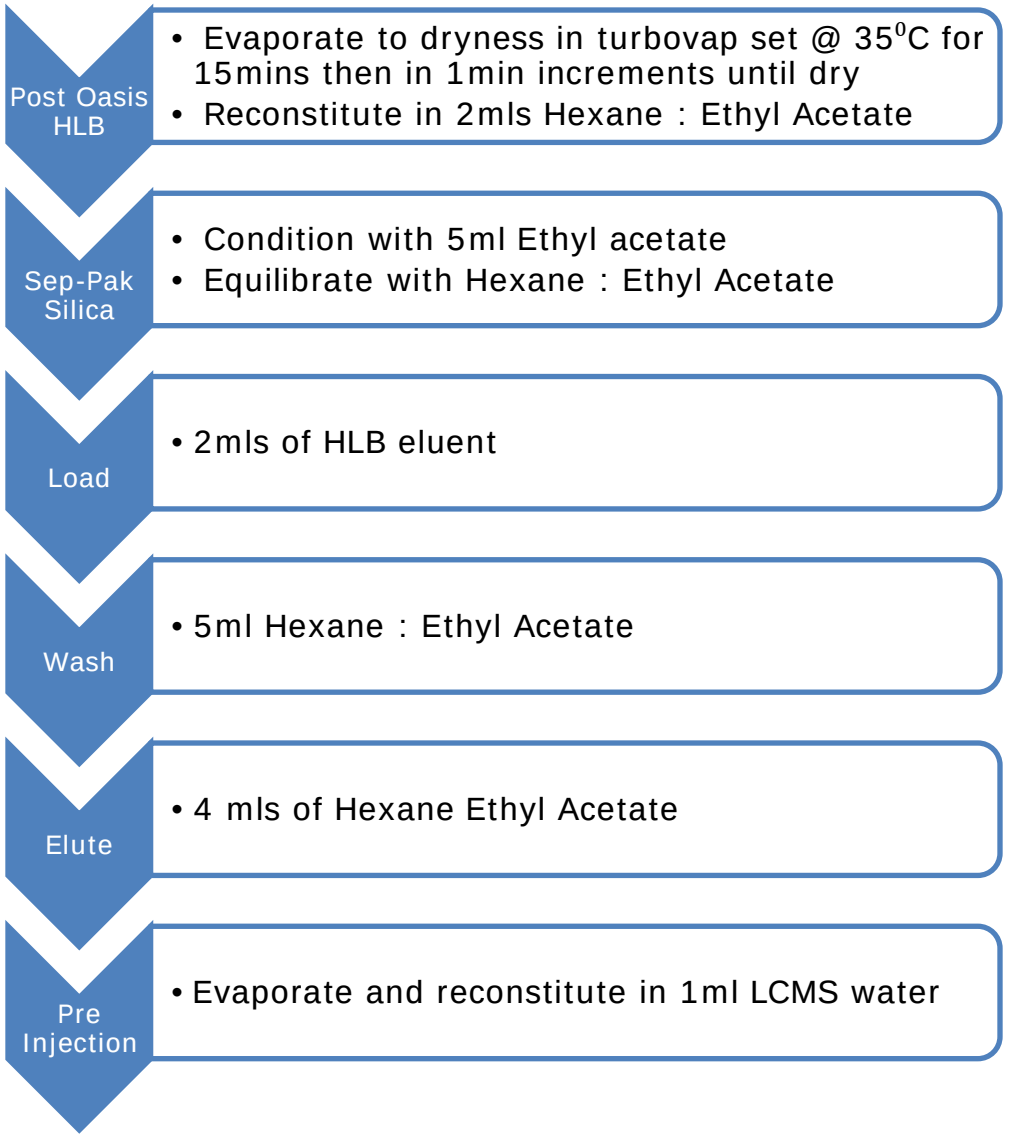
- Effluent Waters
- Sample collected in 1L glass sample bottle (pre treated)
- Sample is then filtered
- 1l of sample is concentrated incorporating wash steps with Oasis HLB and then the HLB eluent evaporated and reconstituted and is applied to Sep-Pak silica.



SPE Average Recoveries

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Compound	Raw Water % Recovery	Final Effluent % Recovery	Crude Influent % Recovery
Estrone	101.8	90.4	92.1
17β-estradiol	103.3	91.7	86.3
17α-ethinylestradiol	104.6	101.7	108.8

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2D LC/MSMS System Parameters

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- 2D ACQUITY Conditions
  - On-Line SPE column – **Oasis HLB direct connect 20µm**
  - Analytical Column – **2.1x100mm ACQUITY BEH C18**
  - Load conditions (BSM Pump A)
    - Injection volume **450ul sample**
    - Loading mobile phase **LCMS water**
  - Elute/Analytical Conditions (BSM Pump B)
    - Elution mobilephase(s), (**A) 0.1% NH4OH (B) 0.1% NH4OH in MeCN**
    - 5 minute gradient **5%-95% (B)**

- XEVO TQ-S Conditions
  - Esi (-) Mode
  - MRM Transitions

Compound	Precursor	Product
Estrone	269.25	159, 145
17 α Ethinyl Estradiol	295.1	158.9, 145
17 β Estradiol	271.2	182.9, 145

ACQUITY UPLC Systems with 2D LC Technology

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Detector

Sample Manager

Column Manager

Pump 1

Pump 2

Configuration – Loading of Sample onto Column 1

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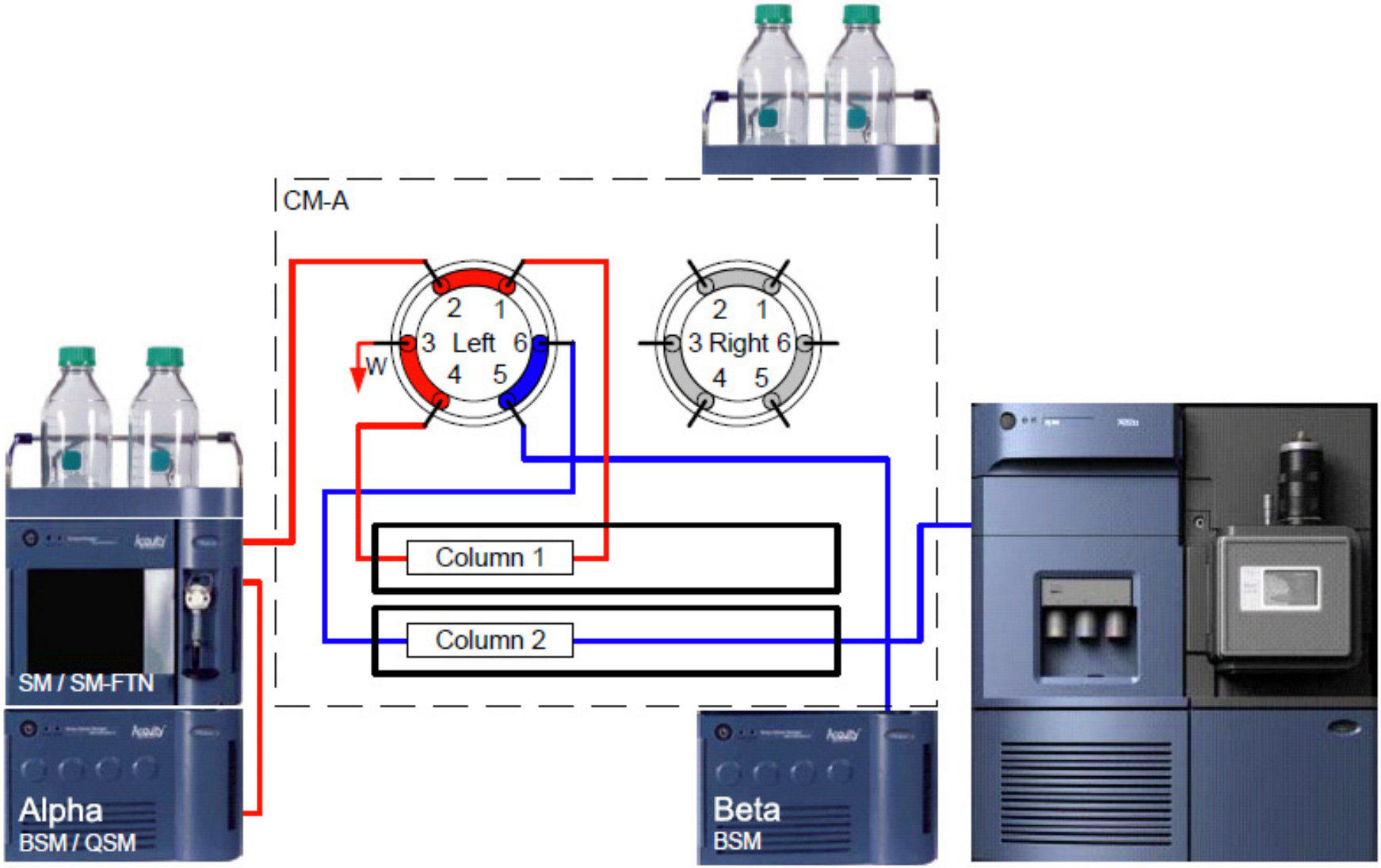

Loading (Trap) Column #1

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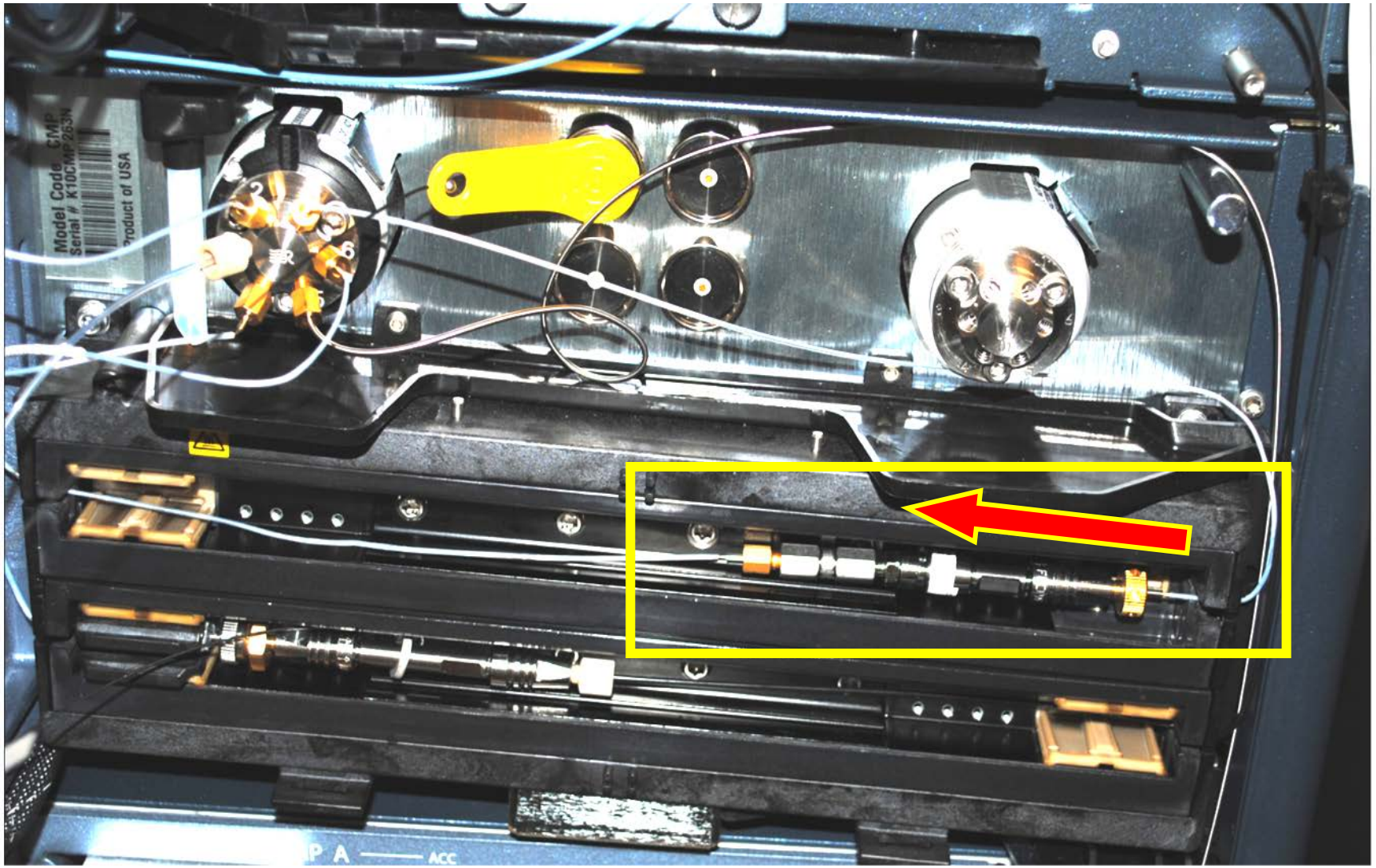

Configuration – Sample Eluted onto Column 2

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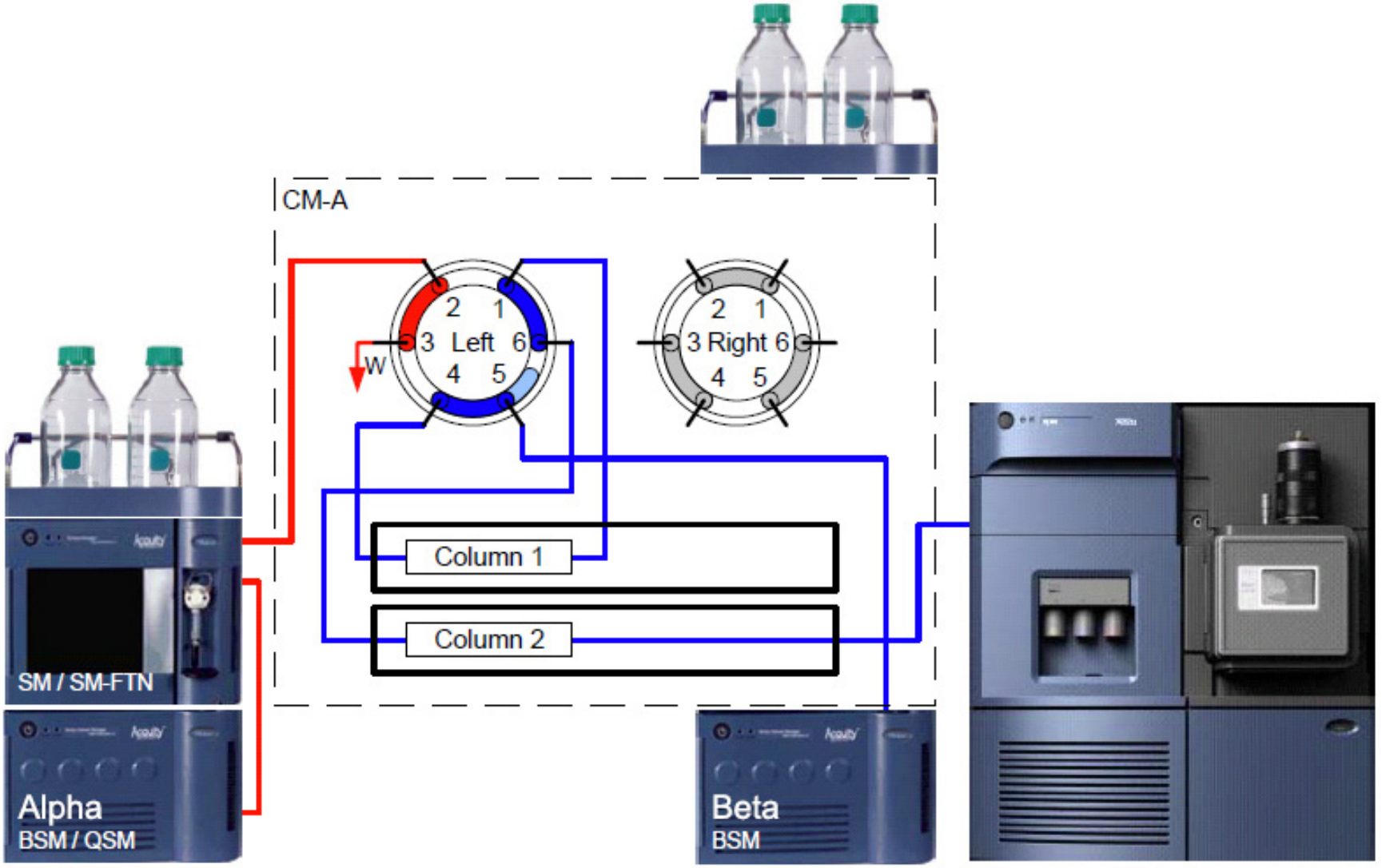

Elute Column #1

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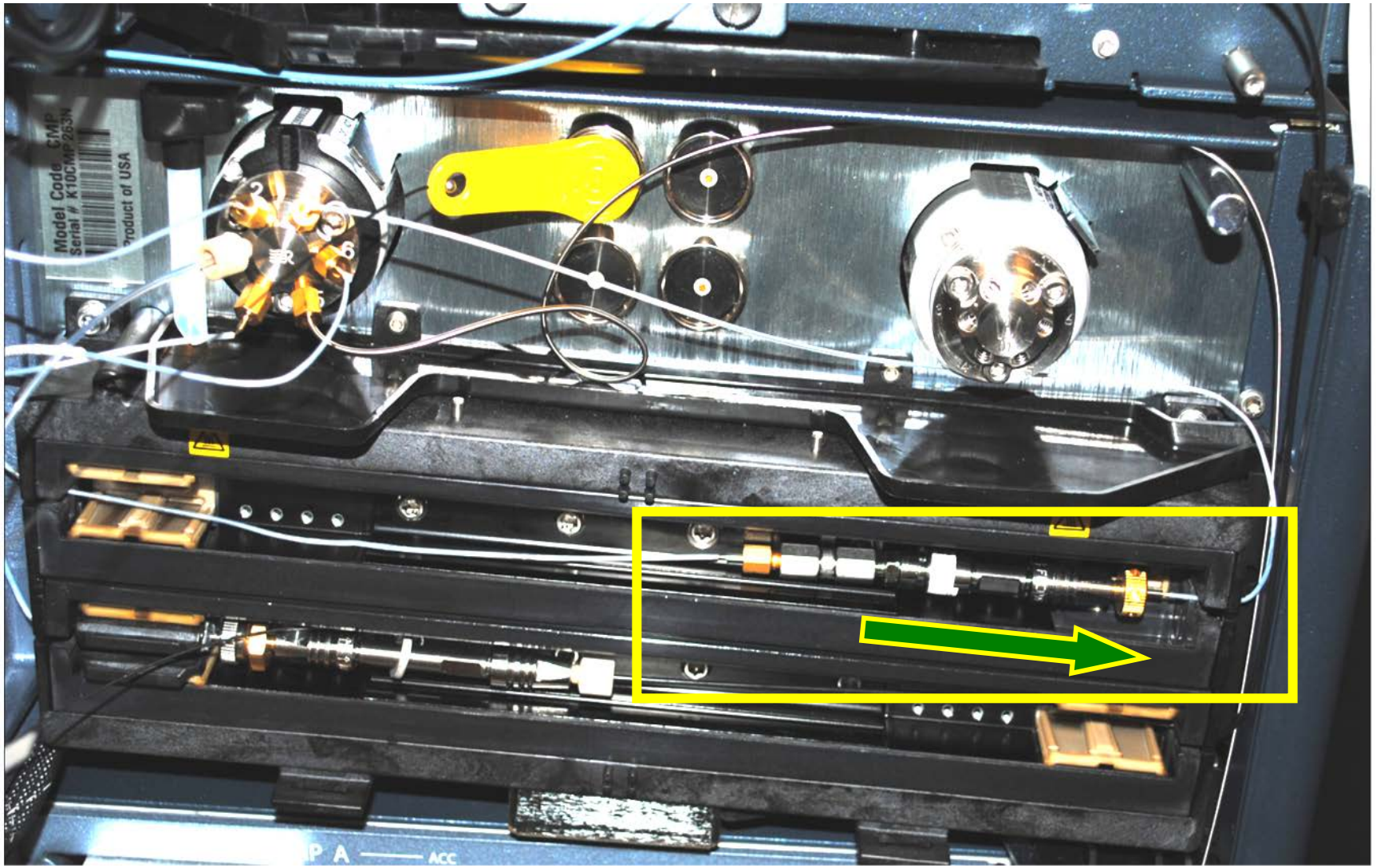

Load onto Column #2

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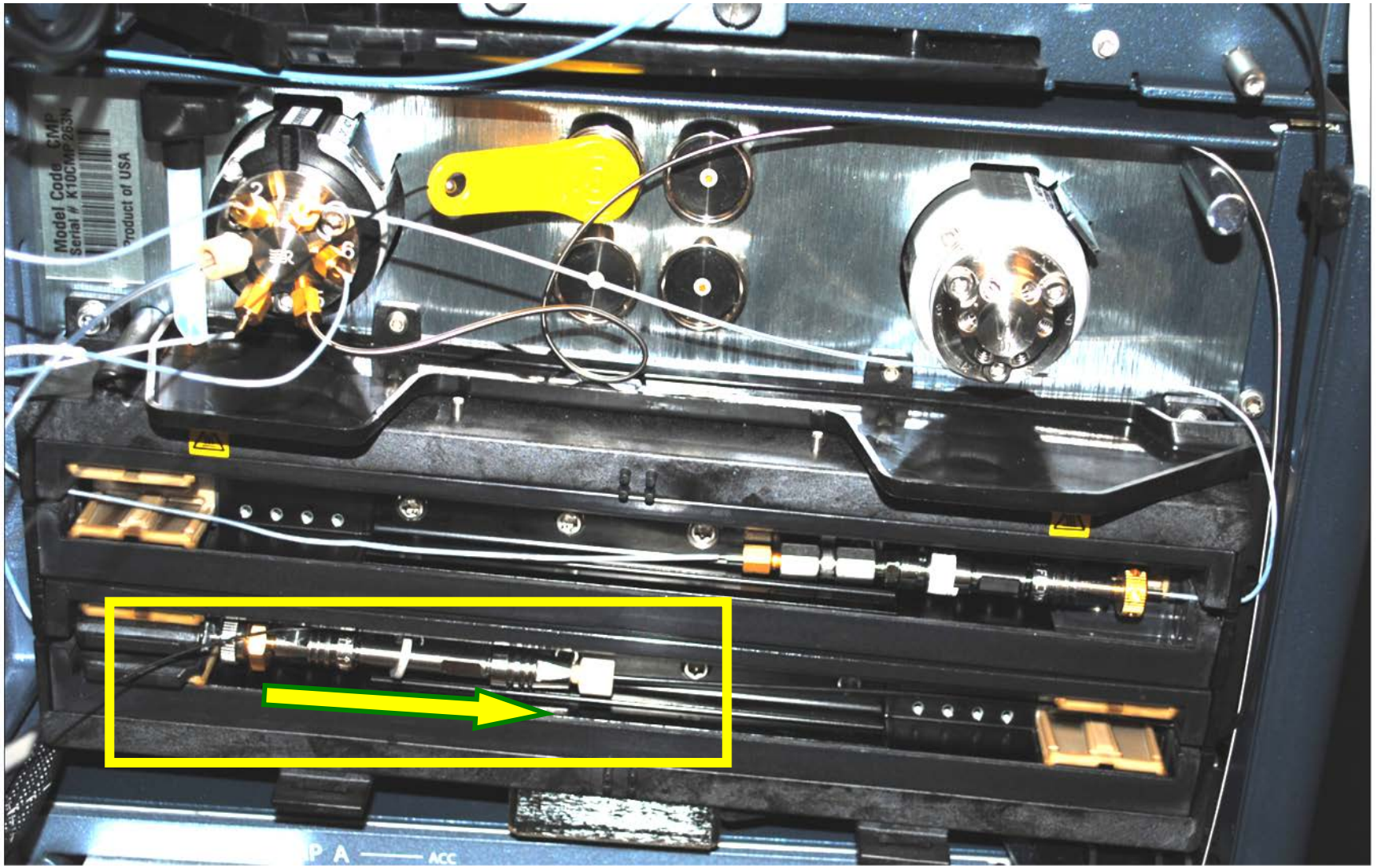

Xevo TQ-S
A *Step* Change in Sensitivity

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Maximising signal

Maximising robustness

Example Chromatography in Elga Water

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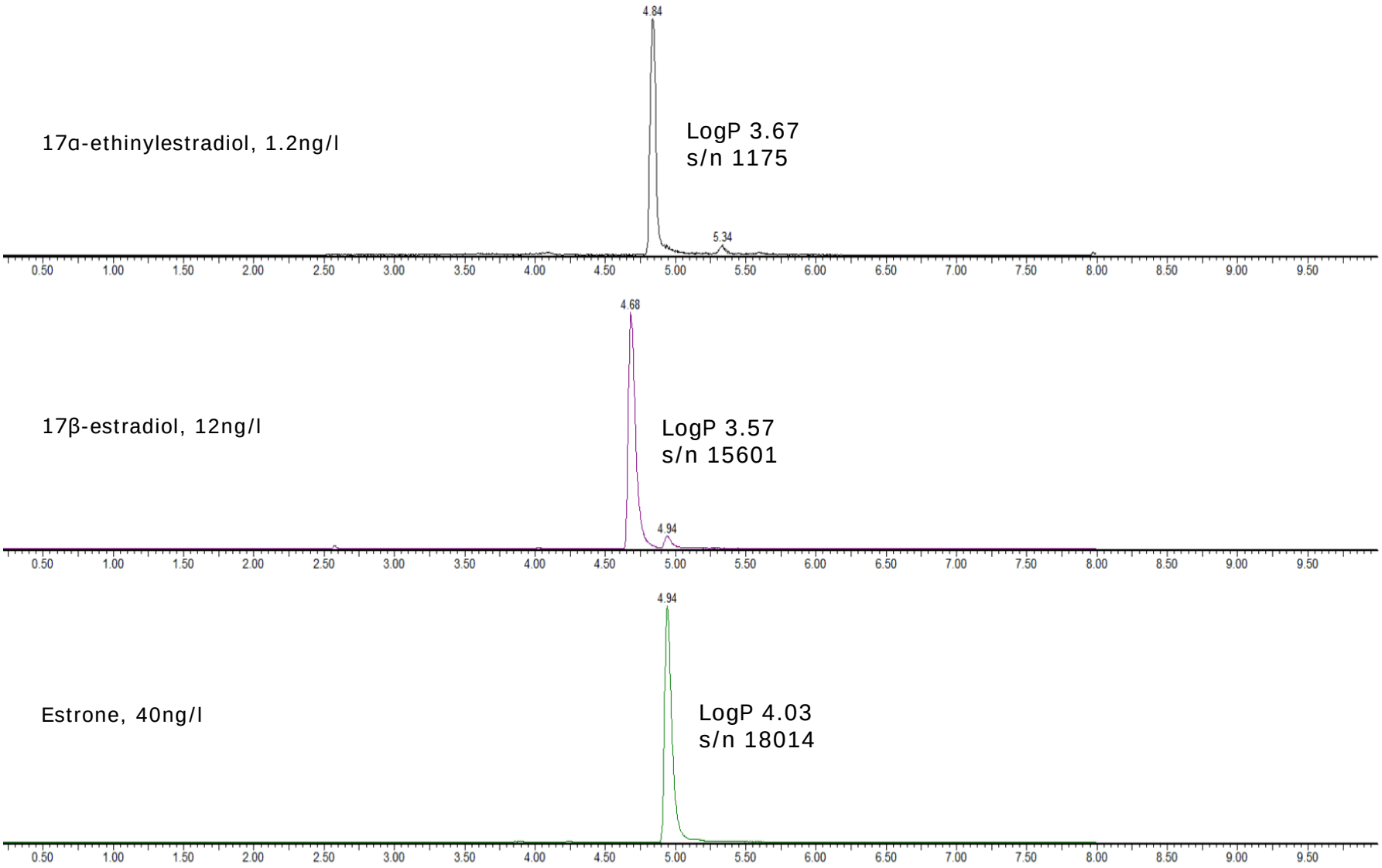

Example Linearity

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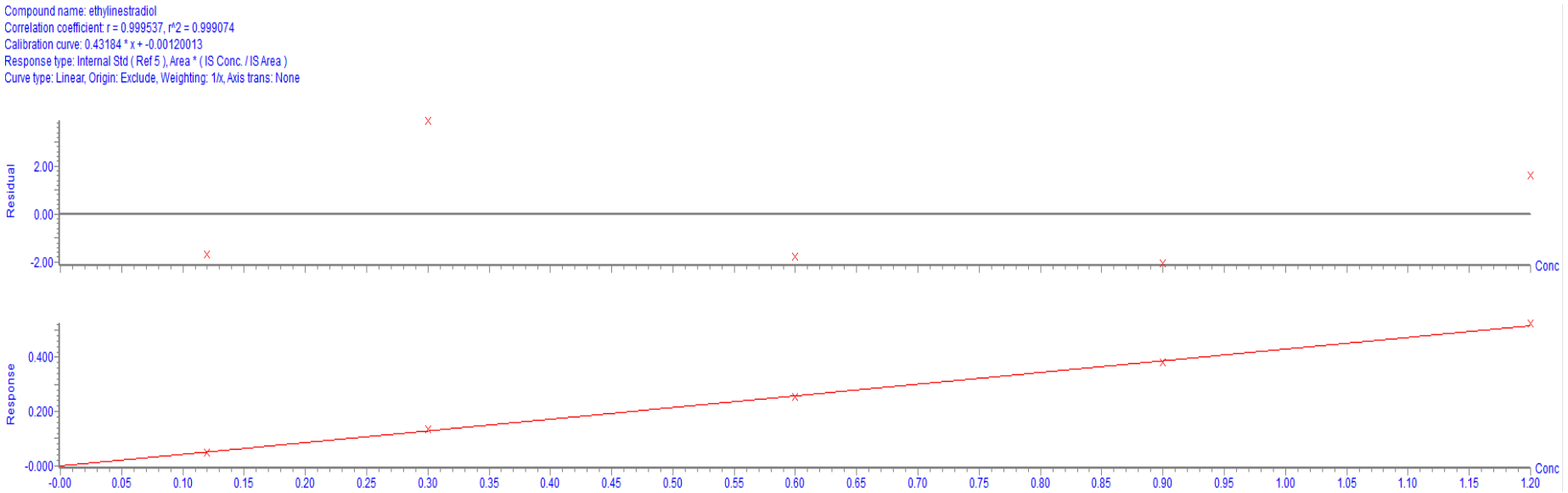

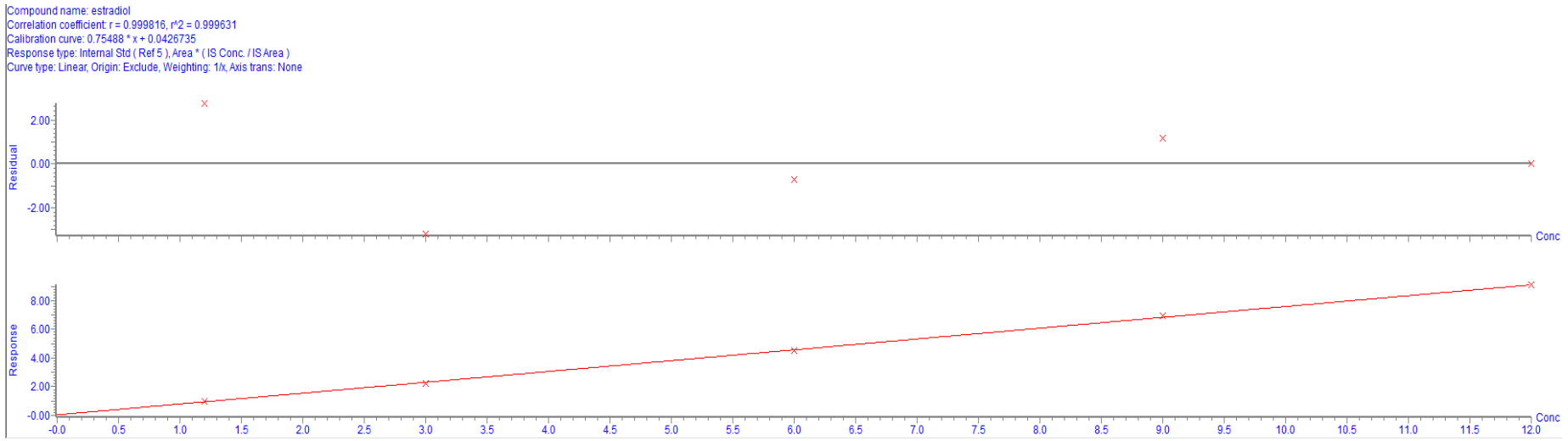

Final effluent from a waste water treatment plant spiked at 0.6ng/l

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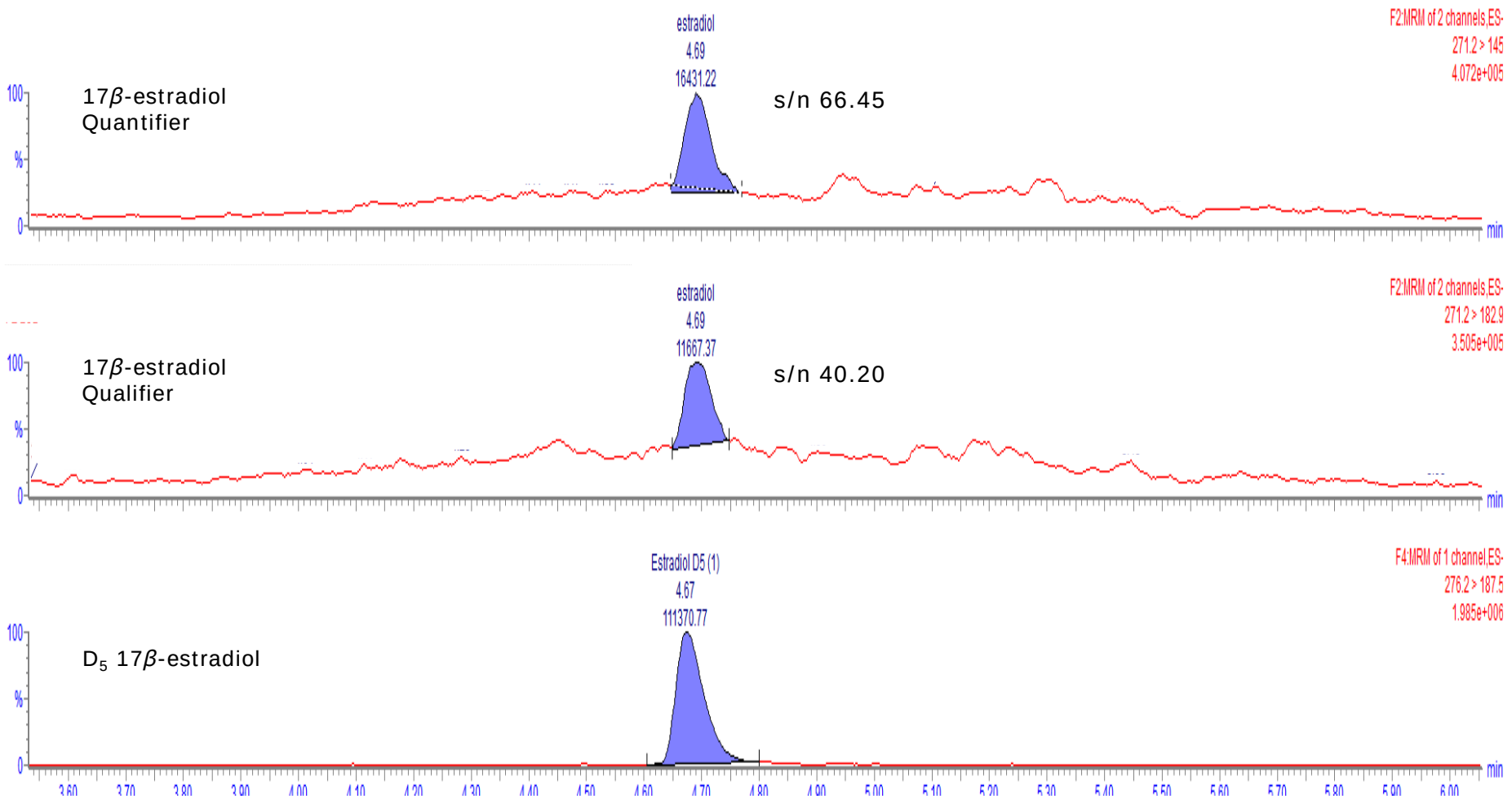

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Summary

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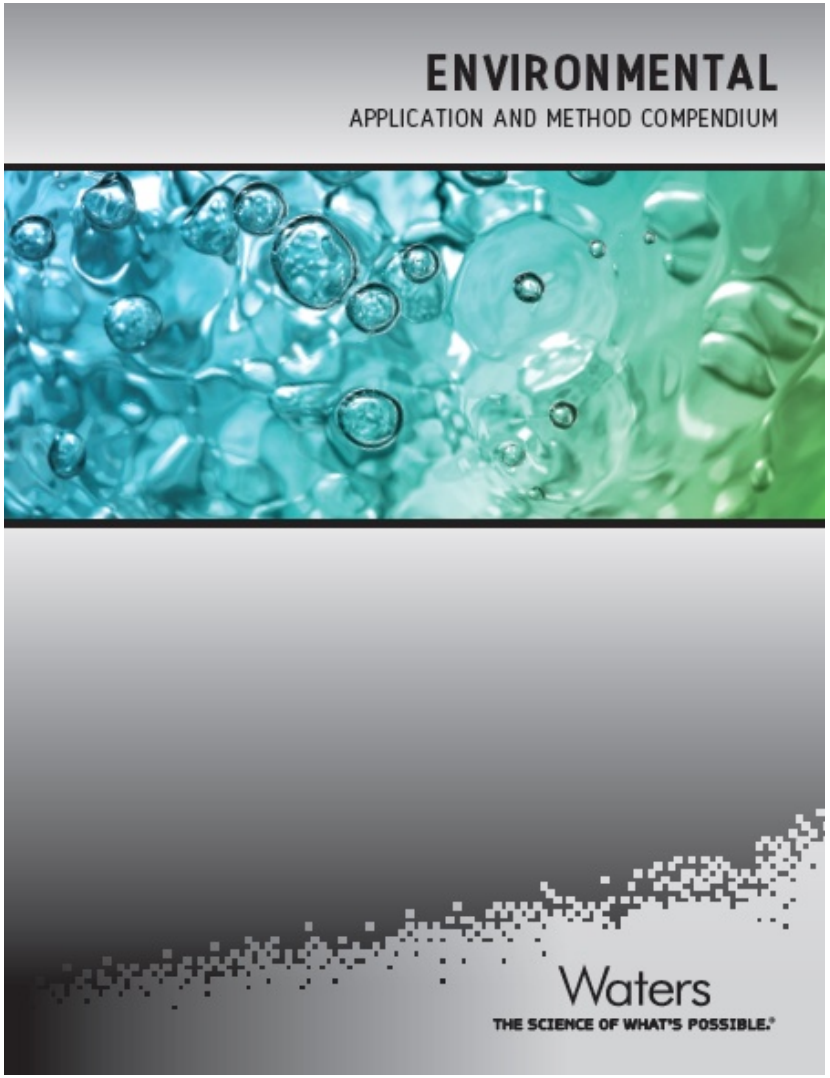
- The combination of off-line SPE followed by analysis on the ACQUITY UPLC system with 2D LC Technology coupled to the XEVO TQ-S allows for ultrasensitive detection of natural and synthetic estrogens in raw water, crude sewage and final effluent water.
- The LOD's for each compound in undiluted matrix are 0.6ng/l for Estrone, 0.3ng/l for 17β Estradiol and 0.03ng/l for 17α Ethinylestradiol.
- The method has undergone a full validation* and was found to meet the required performance criteria.
- **The performance test data comprised of a NS30-style set (NS30, 1989) of tests of eleven batches of duplicate analyses of blanks, low and high standards and low and high spiked samples of effluent. Spiked recovery data was also produced for river and influent matrices*

Acknowledgements



- Scottish Water, Trace Organics, Edinburgh
  - Angela Boag
  - Hamish Todd
  - Neil Gatward
  - Kevin Snaddon