Harmonized GC-Triple Quadrupole Analysis of Steroidal Analogues for Clinical and Environmental Monitoring of the Exposome

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Agilent Technologies

Outline

Epigenetics and the exposome

How do we measure the exposome

The Agilent portfolio in exposomics

17β-estradiol, the environment and cancer

How Agilent can help

Conclusion

May 1, 2013

Genetics, where we are now…

What it has accomplished:

- Elucidation of gene expression and protein function
- Identification biochemical pathways implicated in chronic diseases
- Opportunities for improved treatment and patient management

What it has not accomplished:

- Identify the etiology of 90% of disease

Duke University Medical Center. Retrieved May 10, 2013, from http://www.sciencedaily.com- /releases/2011/01/110123131012.htm

Wild, CP. Cancer Epidemiol Biomarkers Prev 2005;14:1847-1850

Genetics and the environment

E. Callaway. Daily dose of toxics to be tracked. Nature News, Retrieved May 10, 2013 from, http://www.nature.com/news/daily-dose-of-toxics-to-be-tracked-1.11901

Over past 3 decades: unprecedented advances in understanding the genome

The challenge: understand how environment influences genome and shape phenotypes

To address this need, the exposome was defined

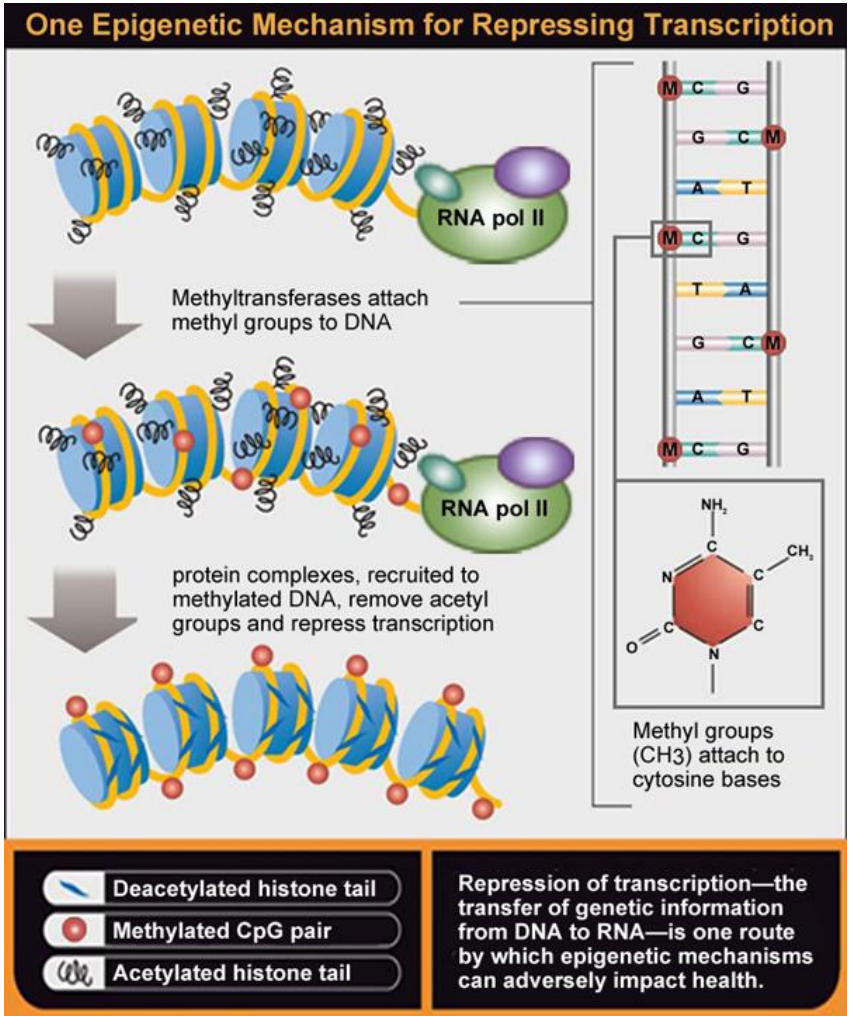
R.A. Stein. Gen Engin Biotechnol News, 33:9 May 1, 2013

Epigenetics

Epigenetic pathways: caused by mechanisms that do not involve mutagenesis

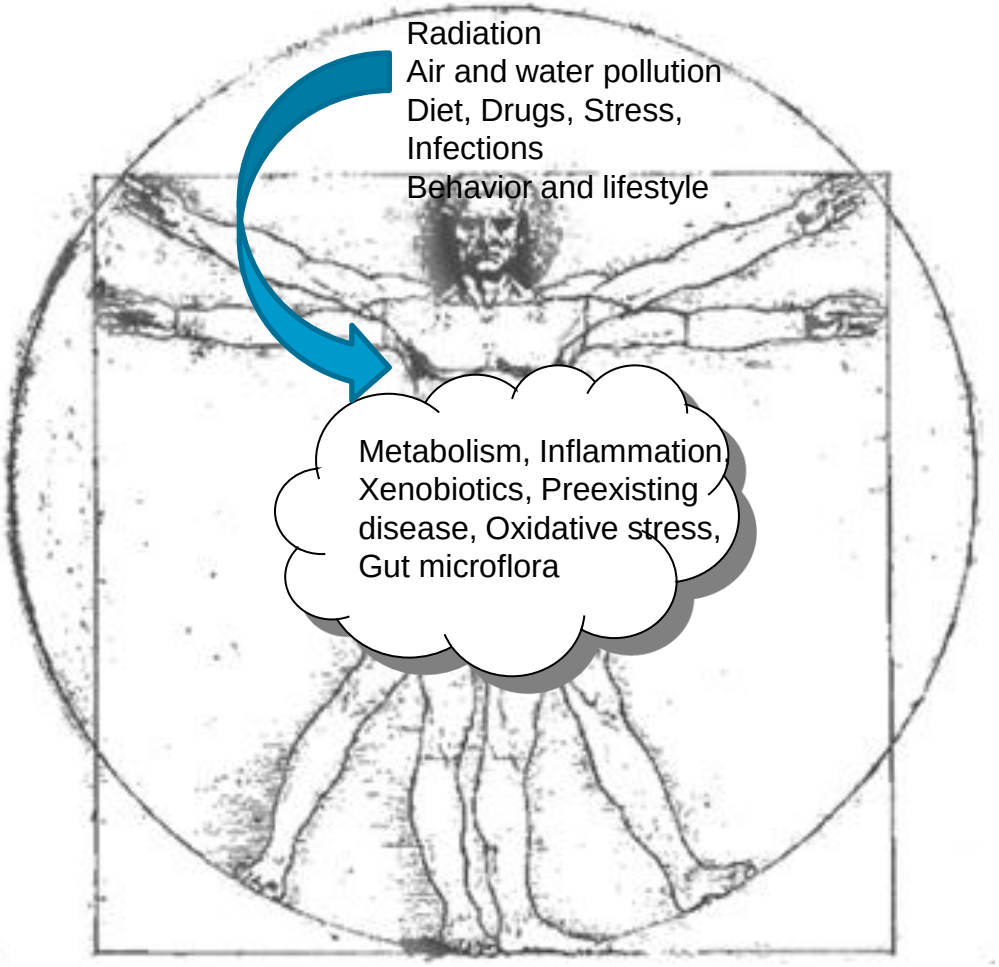
Help us understand the influence of environmental factors

Central to the study of the exposome



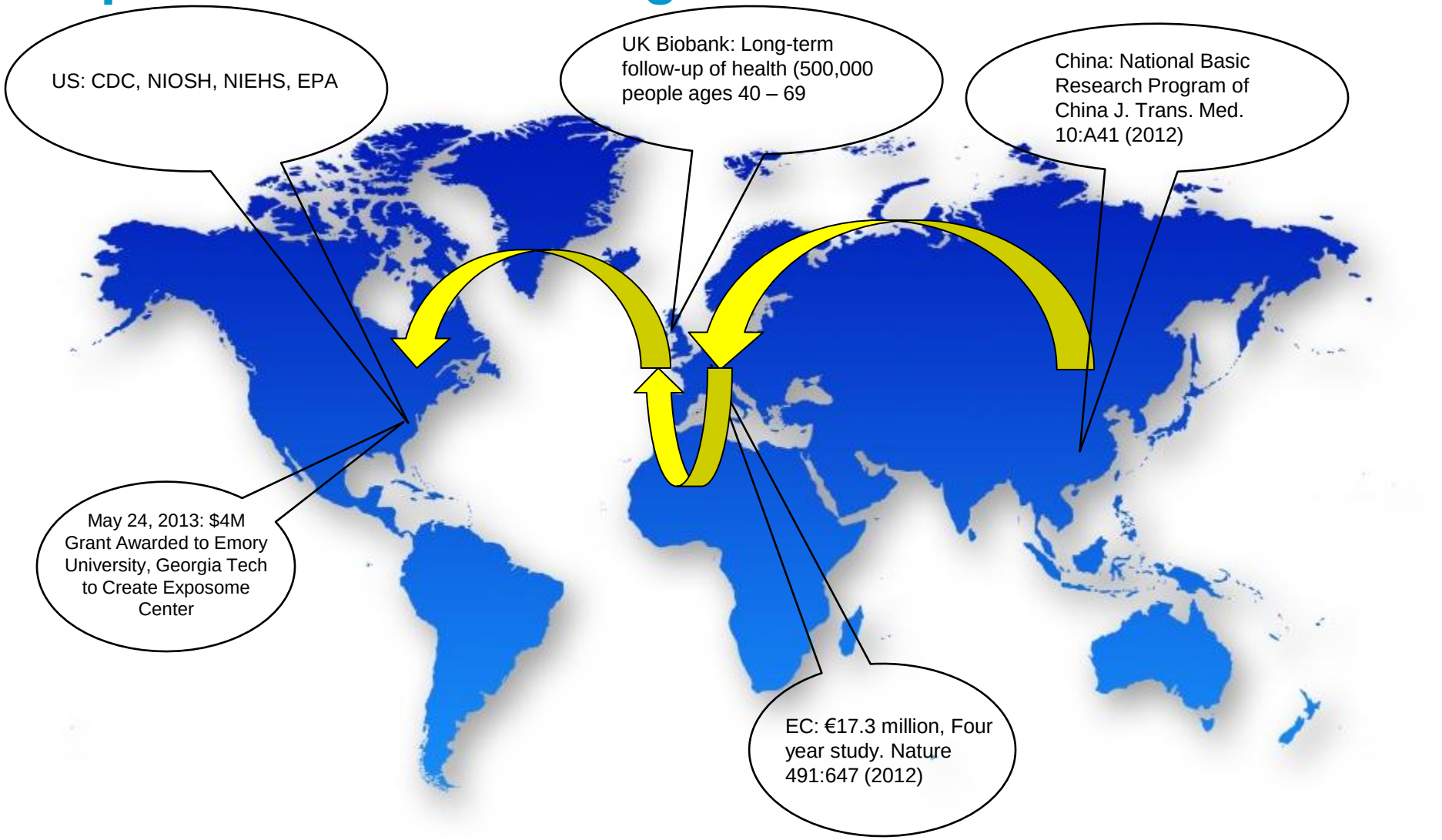
Environmental Epigenetics. National Institute of Environmental Health Sciences. Retrieved May 10, 2013 from, http://www.niehs.nih.gov/research/supported/dert/cospb/programs/envepi/largerview/index.cfm

The exposome & exposomics

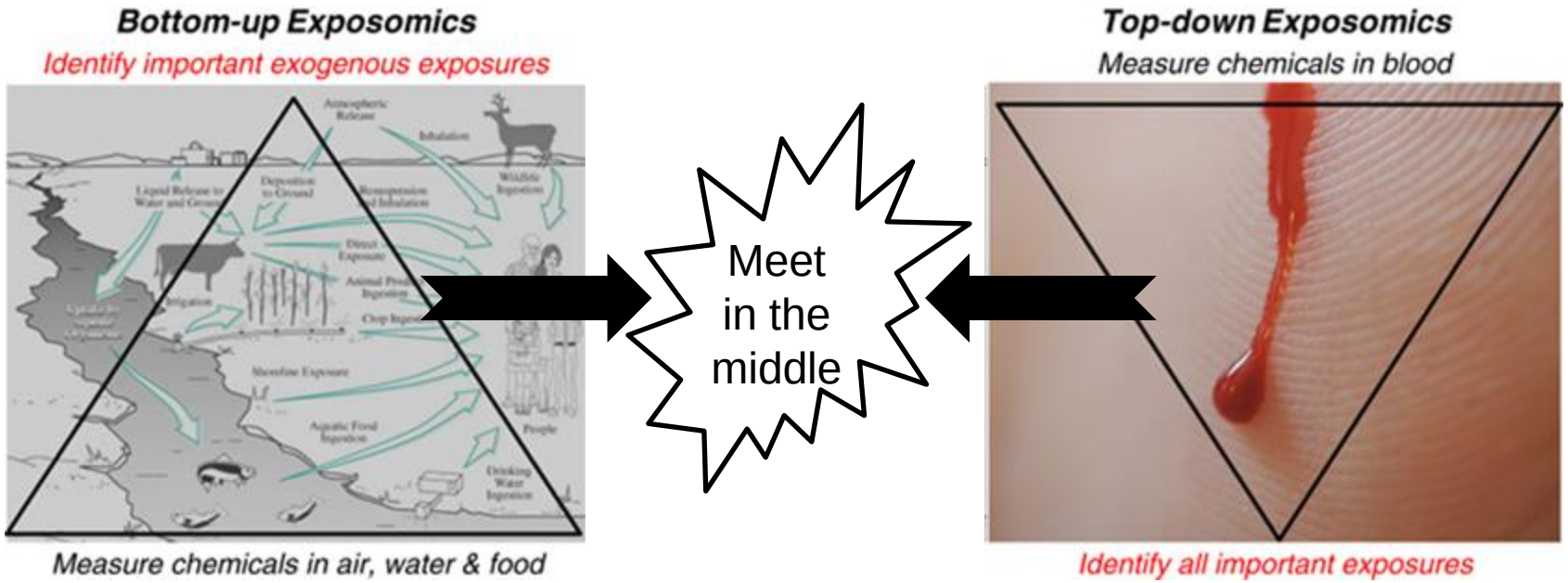


Rappaport SM, J Expo Sci Environ Epidemiol, (2011) 21
Wild CP. Cancer Epidemiol Biomarkers Prev, 2005;14:1847-1850

Exposomics is trending

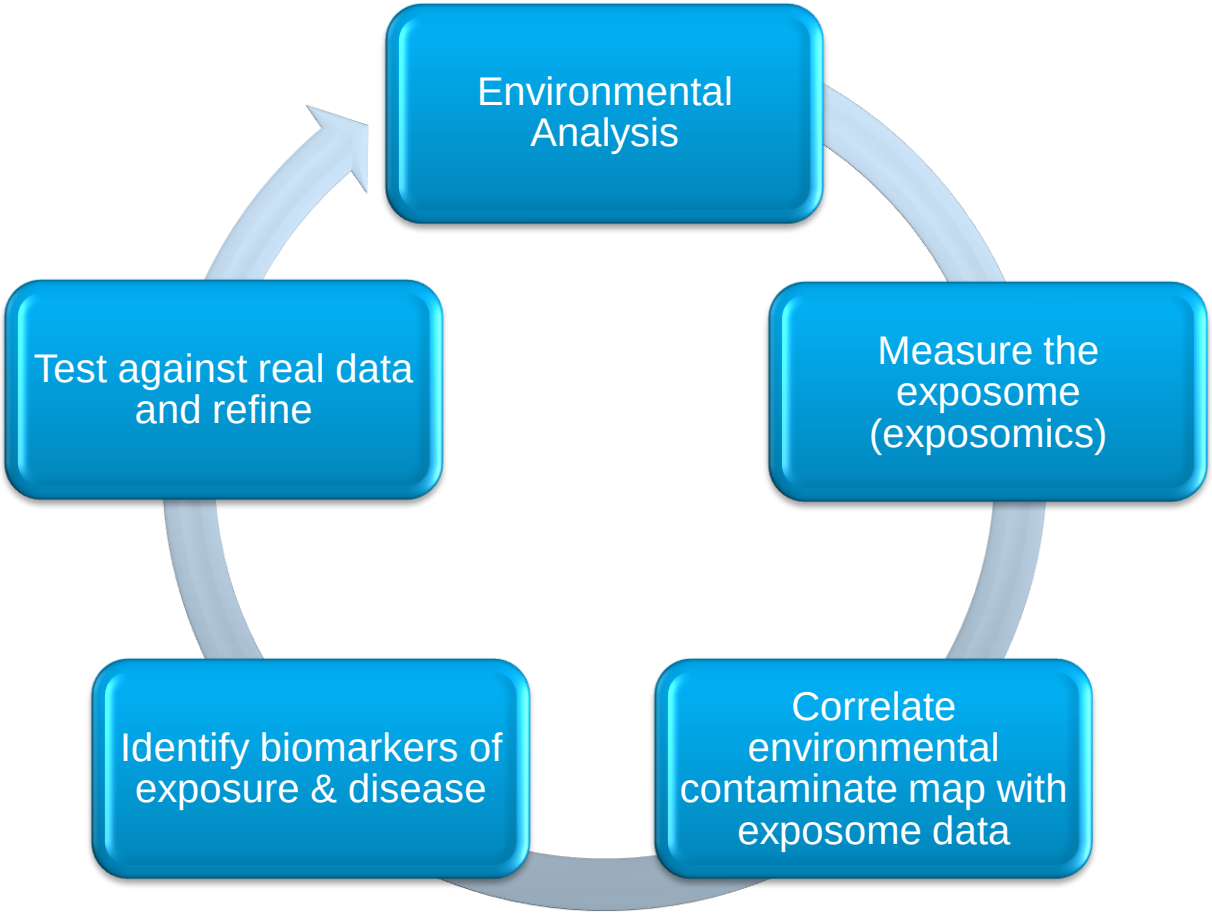


How do we measure the exposome



Rappaport, SM. J Expos Sci Env Epidemiol 2011;21:5–9
M. Chadeau-Hyam, T.J. Athersuch TJ, et al. Biomarkers 2011;16(1):83-88

Cyclical exposomics



A. Macherone. The Future of GC/Q-TOF in Environmental Analysis. In: Advanced Techniques in Gas Chromatography-Mass Spectrometry (GC-MS-MS and GC-TOF-MS) for Environmental Chemistry. I Ferrer & M Thurman (Eds). Elsevier, In Press.

Exposomics, the environment and cancer: 17β-estradiol

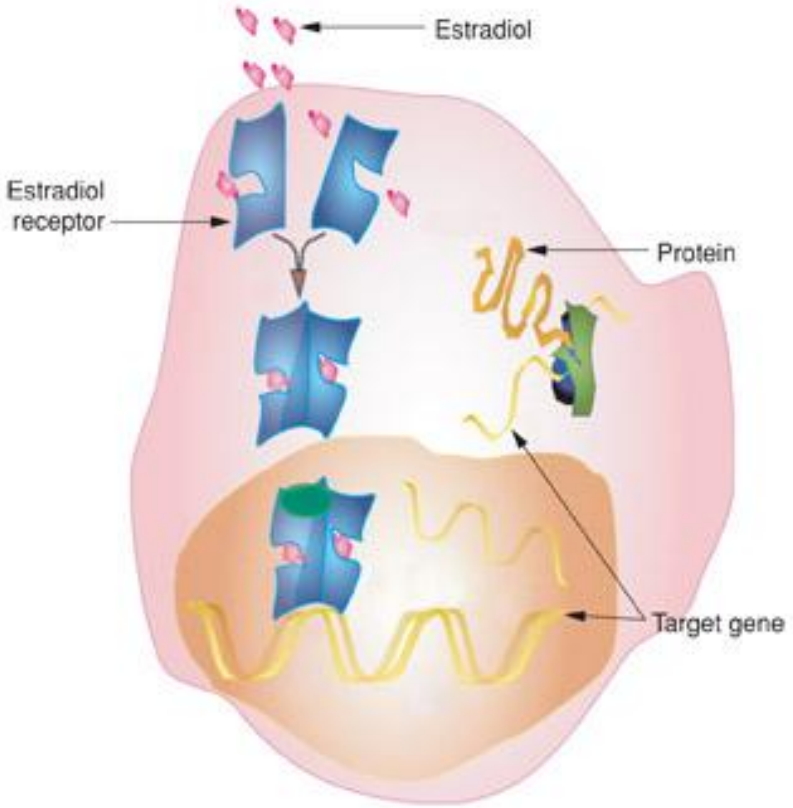
E2 has been determined as persistent environmental pollutant*

- Human and aquatic species

Exposure to E2 via contaminated water has been implicated in estrogen induced breast cancer

Epigenetics:

– *The Stimulation of HSD17B7 Expression by Estradiol Provides a Powerful Feed-Forward Mechanism for Estradiol Biosynthesis in Breast Cancer Cells**



G. Rennie. Exploring the link between the diet and cancer. Science & Technology. Retrieved May 10, 2013 from, https://www.llnl.gov/str/December05/Kulp.html

*A. Macherone, M. Churley. Monitoring steroidal analogues in clinical and environmental chemistry: one model for exposomics. ASMS poster 2013.
**S.A. Albarracin, S.Y. Devi, et al. Mol Endocrinol, May 2011, 25(5):754–766.

Purpose of study

Proof of concept

- Develop harmonized analytical method to measure serum estrogen and exogenous estrogen exposome
  - Reciprocal top-down / bottom-up approach
  - Robust, high specificity, extremely sensitive (< 1.0 pg/mL)
  - Correlate *in vitro* estrogen levels to that of environmental data
  - Utilize bioinformatics and multi-variant analysis e.g., Mass Profiler Professional

Study design

Targeted measurement of a region

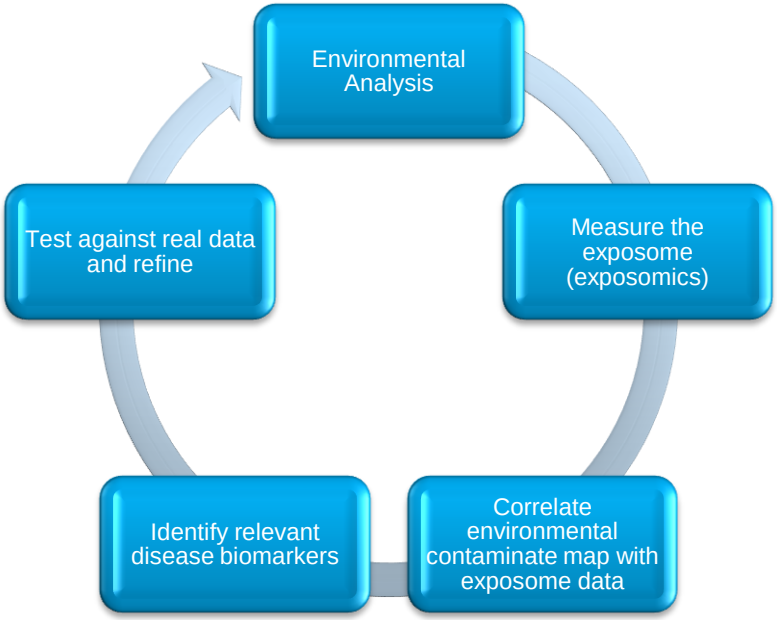
Exposome measurement of the regions inhabitants

Correlation of source data, disease history

- Public health information or baseline questionnaires

Align regional and exposome data

Correlate biomarkers of exposure / biomarkers of disease



Biological and Environmental Monitoring of 17β-Estradiol

METHOD

GC/MS/MS method

Agilent 7890A 7000B GC/MS/MS system

Multi Mode Inlet, 2 µL injection volume

Two DB-17ht columns, He constant flow mode

- 5m x 0.25mm x 0.15µm
- 15m x 0.25mm x 0.15 µm

Purged Ultimate Union used for back flushing the column

Oven program 90 to 330°C

Negative chemical ionization

40% ammonia reagent gas

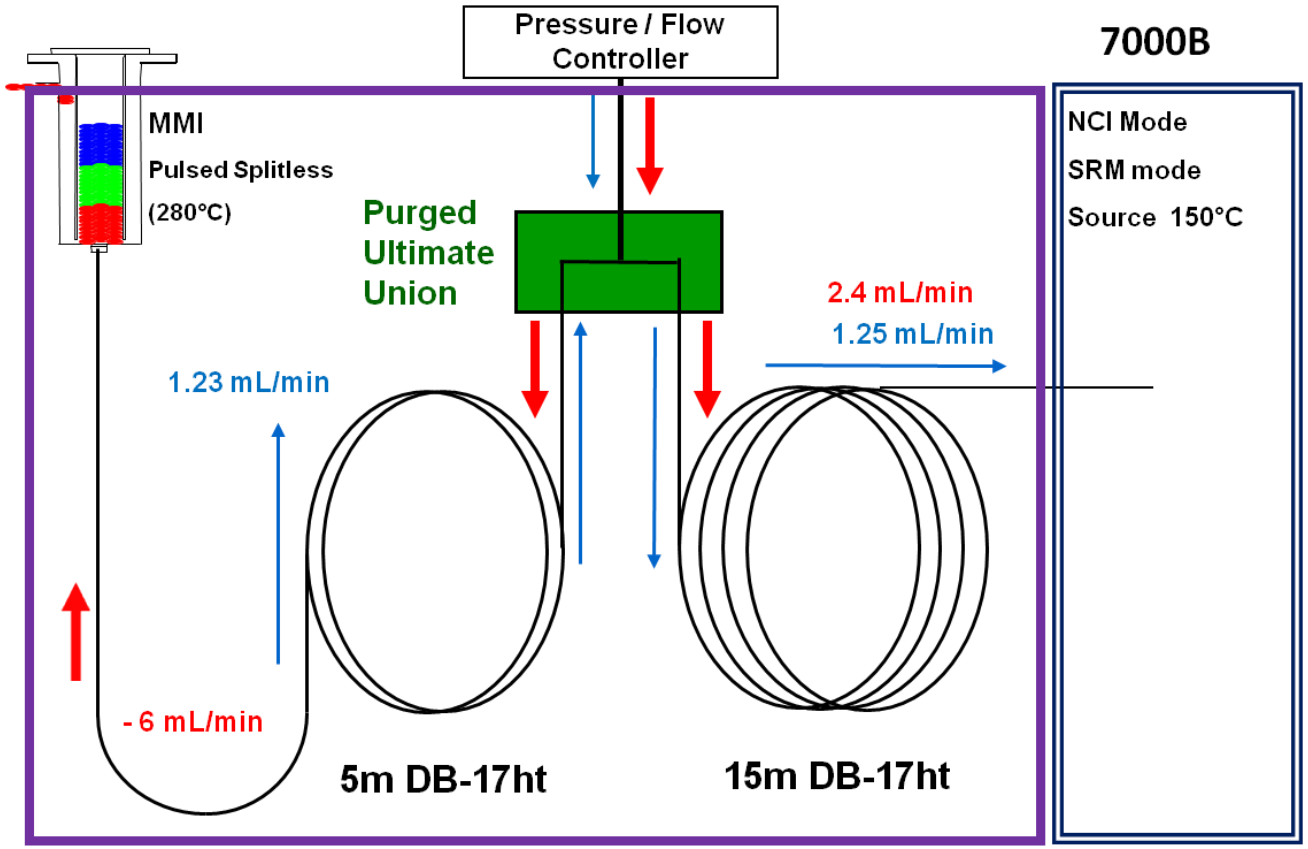
Source temperature 150°C

Transfer line 310°C

SRM transitions

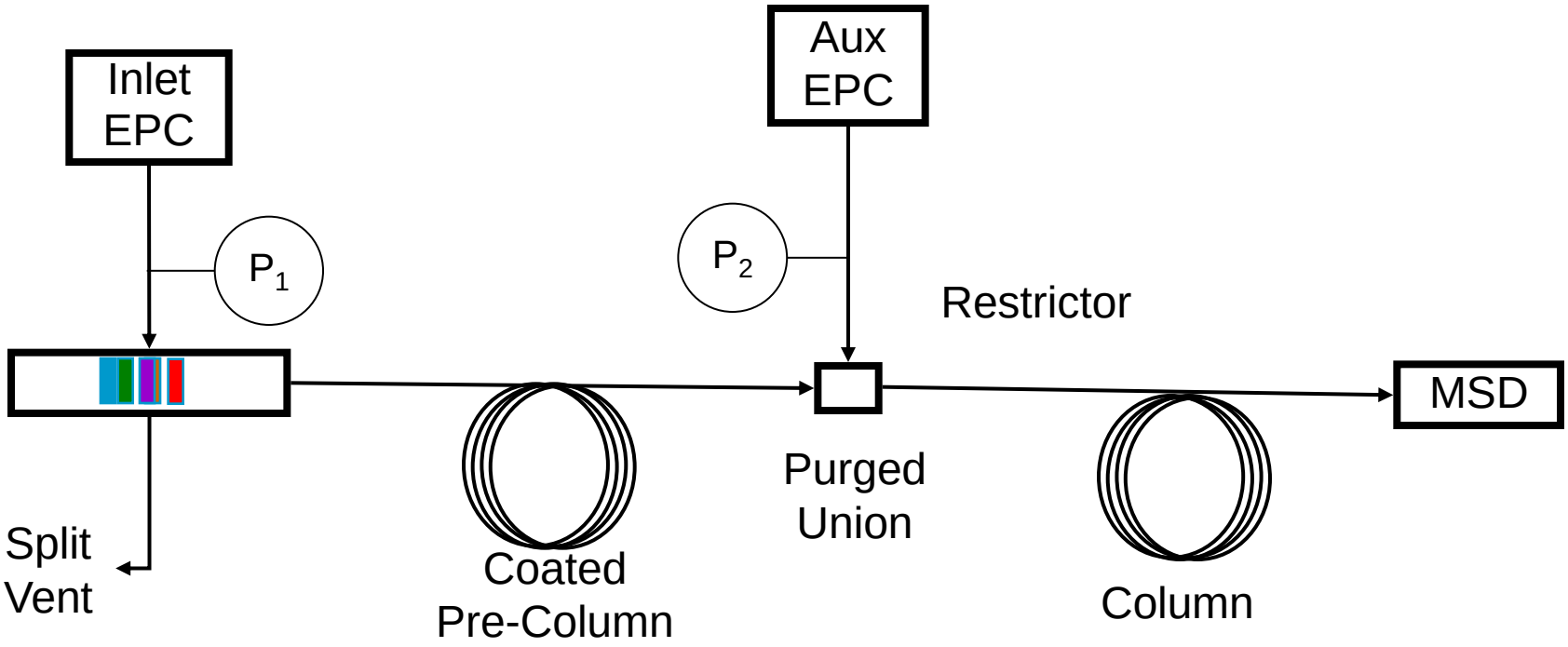
Time Segment	Compound	Precursor Ion	Product Ion	dwell	Collision Energy
1	EE2	490.5	426.5	60	6
1	E1	464.4	400.4	60	6
1	EQ	462.4	398.4	60	4
1	EQ	462.4	370.4	60	8
1	ASD	461.5	431.5	60	6
2	Testosterone	677.6	657.6	100	4
2	Testosterone	677.6	627.6	100	6
2	E2	660.5	596.5	100	8
3	E3	870.6	806.6	150	6
3	E3	870.6	167.6	150	10

Column configuration

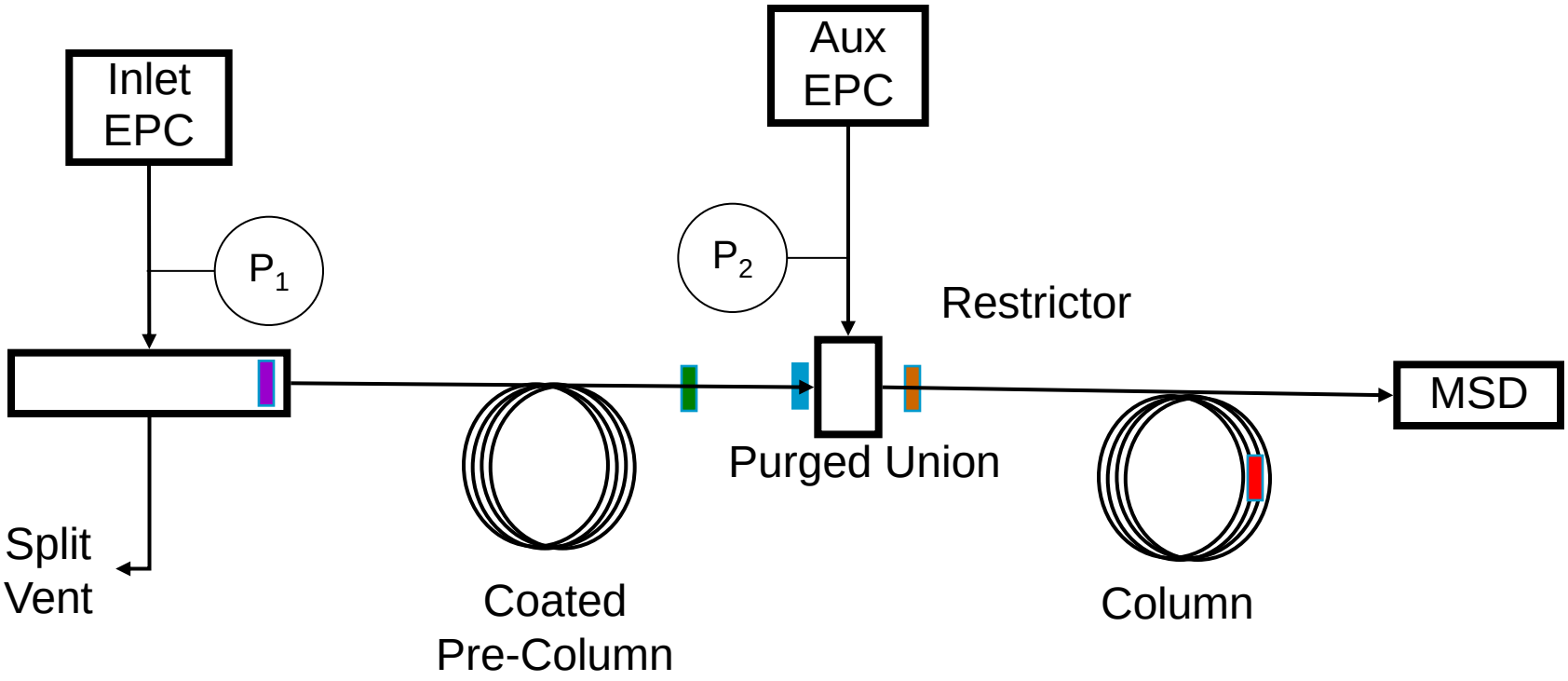


Blue – Analysis
Red – Back Flush

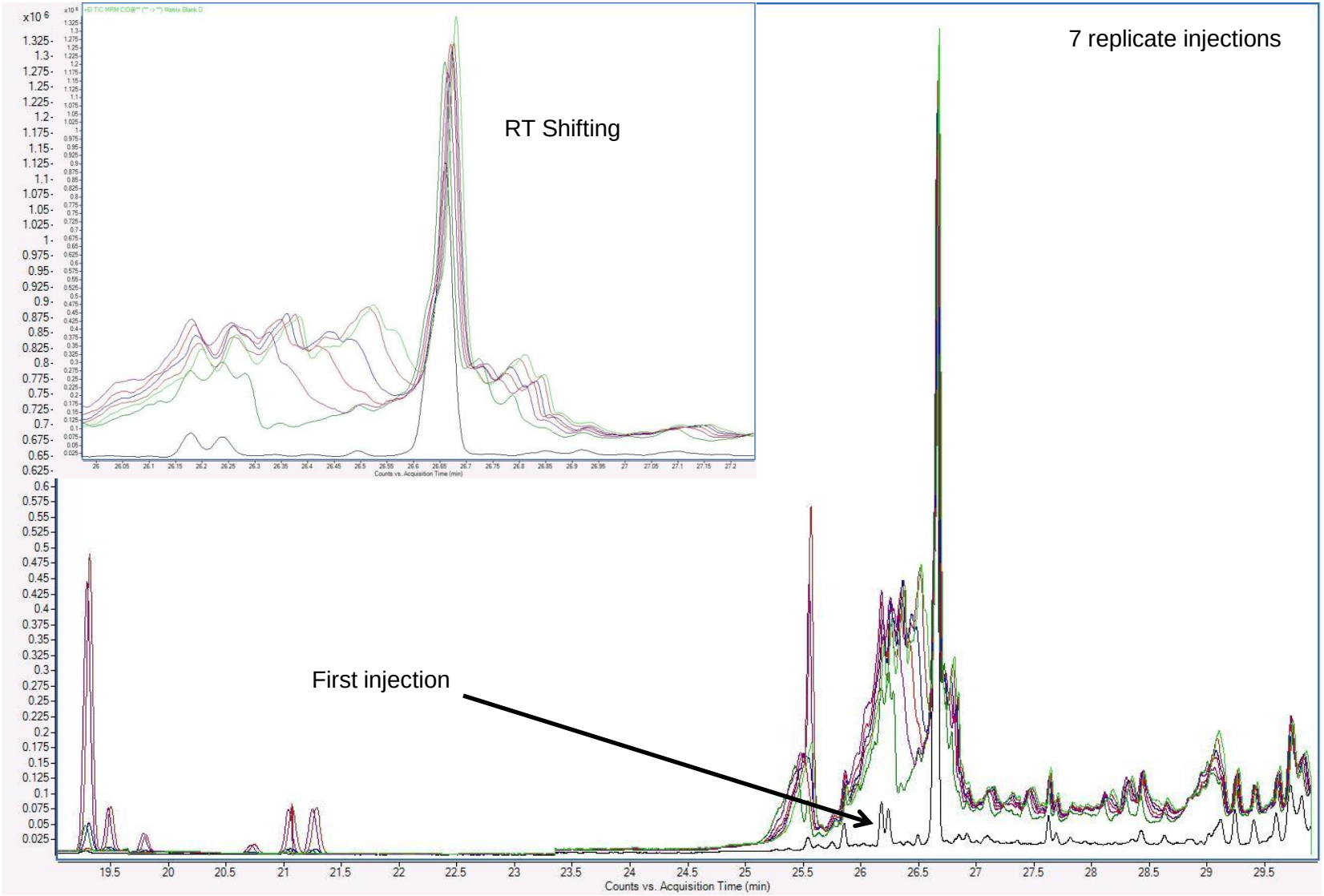
Pre-Column BF Forward Flow



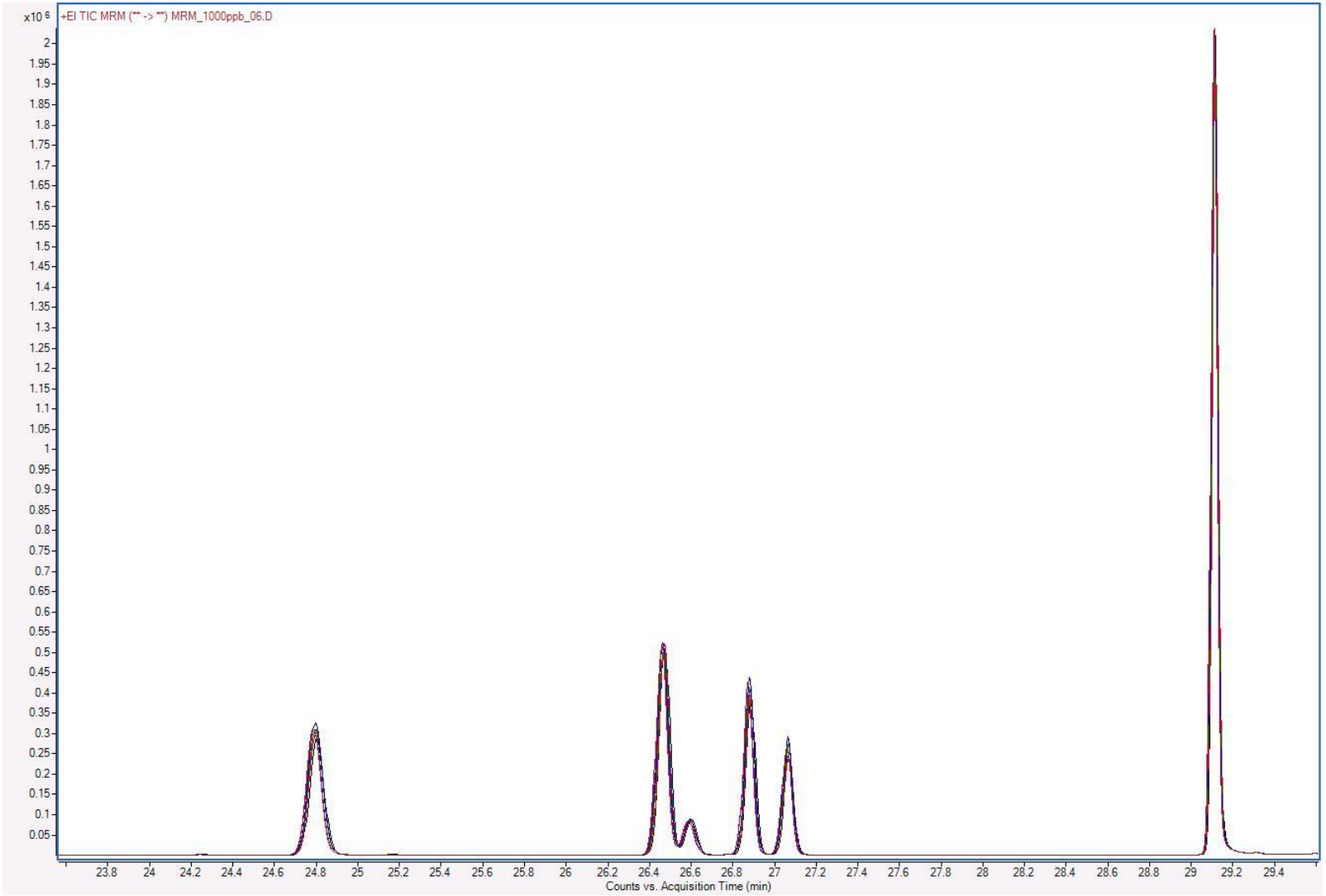
Pre-Column Concurrent Backflush



No BF


7 replicate injections
RT Shifting
First injection
Counts vs. Acquisition Time (min)

With BF


x10 6
+EI TIC MRM (** -> **) MRM_1000ppb_06.D
2
1.95
1.9
1.85
1.8
1.75
1.7
1.65
1.6
1.55
1.5
1.45
1.4
1.35
1.3
1.25
1.2
1.15
1.1
1.05
1
0.95
0.9
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
23.8
24
24.2
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24.6
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25
25.2
25.4
25.6
25.8
26
26.2
26.4
26.6
26.8
27
27.2
27.4
27.6
27.8
28
28.2
28.4
28.6
28.8
29
29.2
29.4
Counts vs. Acquisition Time (min)

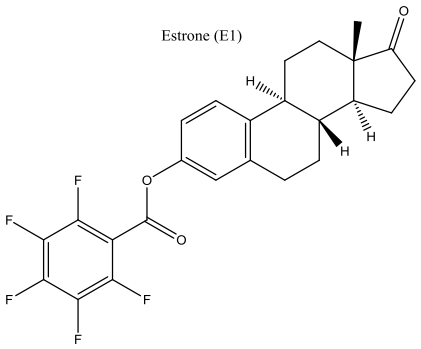
Derivatization

One-step derivatization

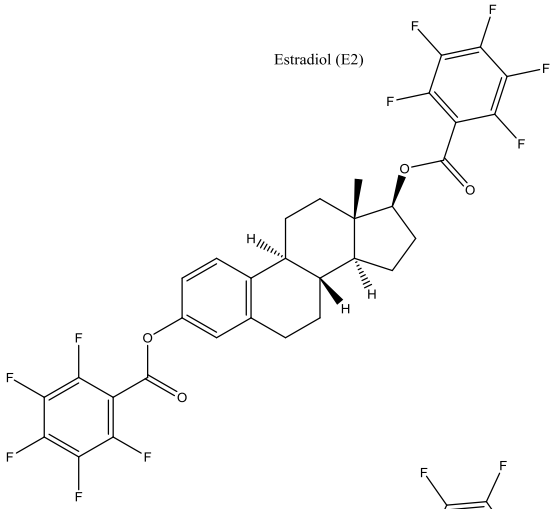
- Synthesize pentafluorobenzoyl (PFB) ester
- The PFB ester is formed at C-17 in some cases and also at C-16 in the case of E3

Derivatized estrogens

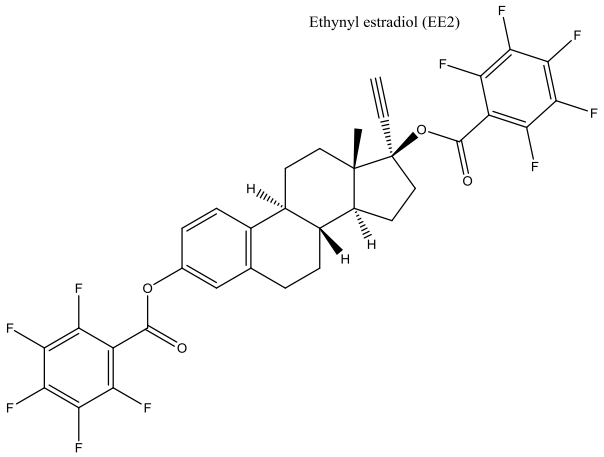
Estrone (E1)



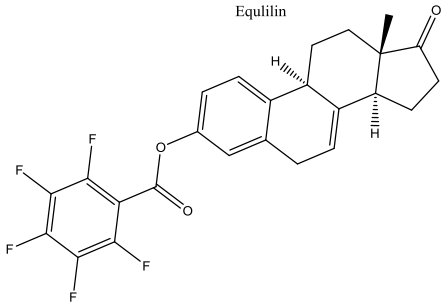
Estradiol (E2)



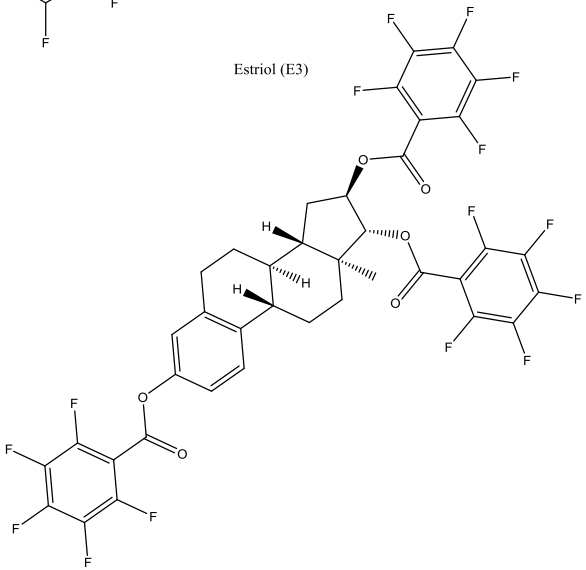
Ethynyl estradiol (EE2)



Equlilin



Estriol (E3)



Samples and Calibrators

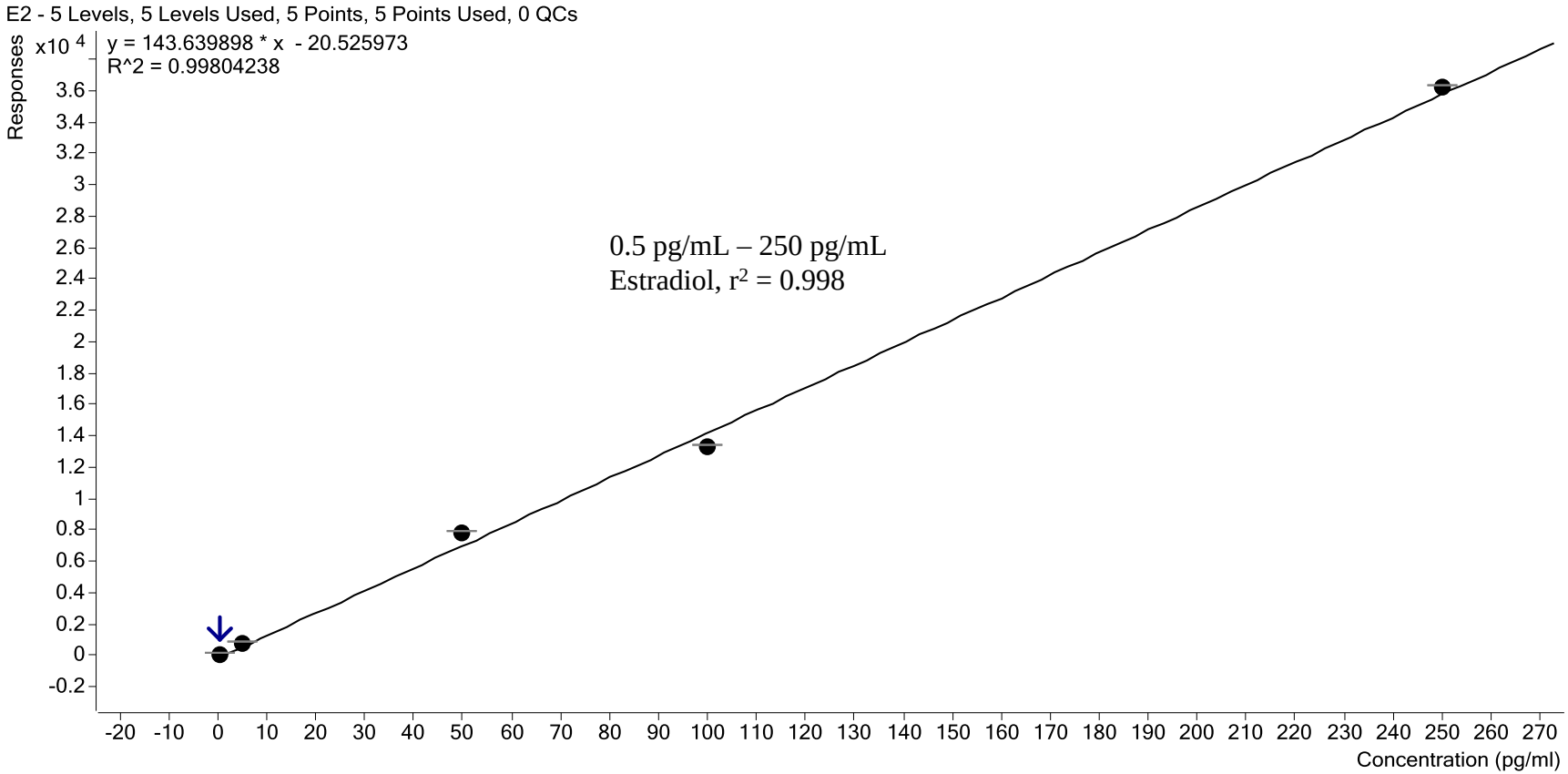
- ✓ Water: Calibration levels ranging from 0.05 pg/mL to 250 pg/ml
- ✓ Serum: Calibration levels ranging from 1.0 pg/mL to 600 pg/ml
- ✓ ISTD: Estradiol-D3, Testosterone-D5, Estrone-D4 at 10 pg/ml

  - Water sample volume = 20 mL
  - Serum sample volume = 0.4 mL

Biological and Environmental Monitoring of 17β-Estradiol

RESULTS

E2 Water Calibration Curve



Instrument Detection Limit (IDL): Water extracts

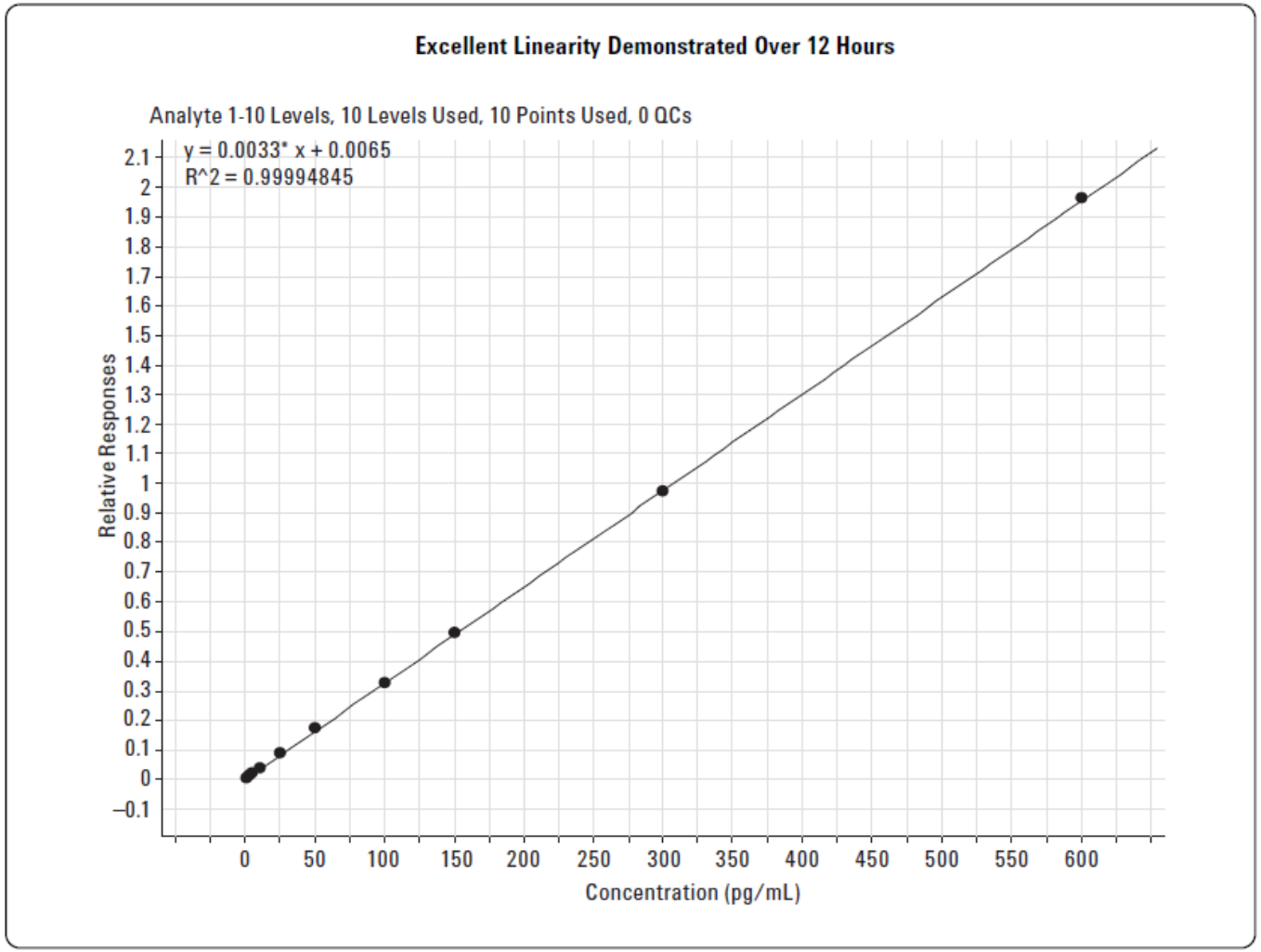
Determined by the equation:
$IDL = (t_{\alpha})(\%RSD_{area})(\text{conc. of standard})/100$

where t_{α} is a statistical confidence factor found in the Student t- distribution table. With 99% confidence, the t_{α} value for 5-1 degrees of freedom is 3.747

Substituting this into the equation:
IDL = (3.747)(6.8%)(0.5 pg/ml)/100 =
0.13 pg/ml E2

Name	E2 Area
0p5_01.D	684
0p5_02.D	620
0p5_03.D	723
0p5_04.D	641
0p5_05.D	620
Average	658
St Dev	45
% RSD	6.8

E2 linearity (serum)



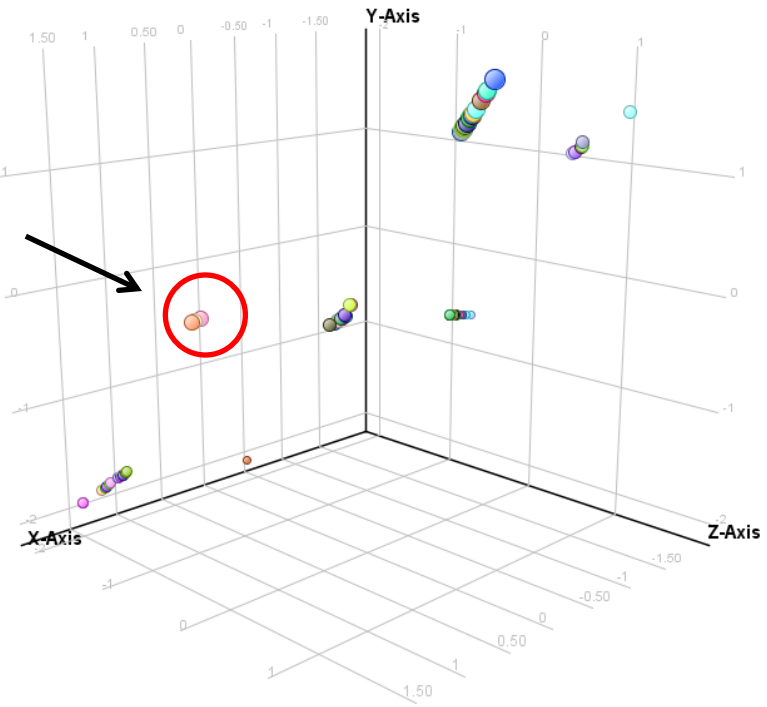
E2 Replicate injections (serum): IDL = 0.41 pg/mL (recently reported at 0.12 pg/mL*)

Sample Name	Concentration (pg/mL)	N	Response Ratio
EA-1-1	1.0	1	0.0071
EA-1-2	1.0	2	0.0087
EA-1-3	1.0	3	0.0086
EA-1-4	1.0	4	0.0087
EA-1-5	1.0	5	0.0078
EA-1-6	1.0	6	0.0076
EA-1-7	1.0	7	0.0047
EA-1-8	1.0	8	0.0085
EA-1-9	1.0	9	0.0078
EA-1-10	1.0	10	0.0079
		Average	0.0077
		Std. Dev.	0.0011
		% RSD	14.69

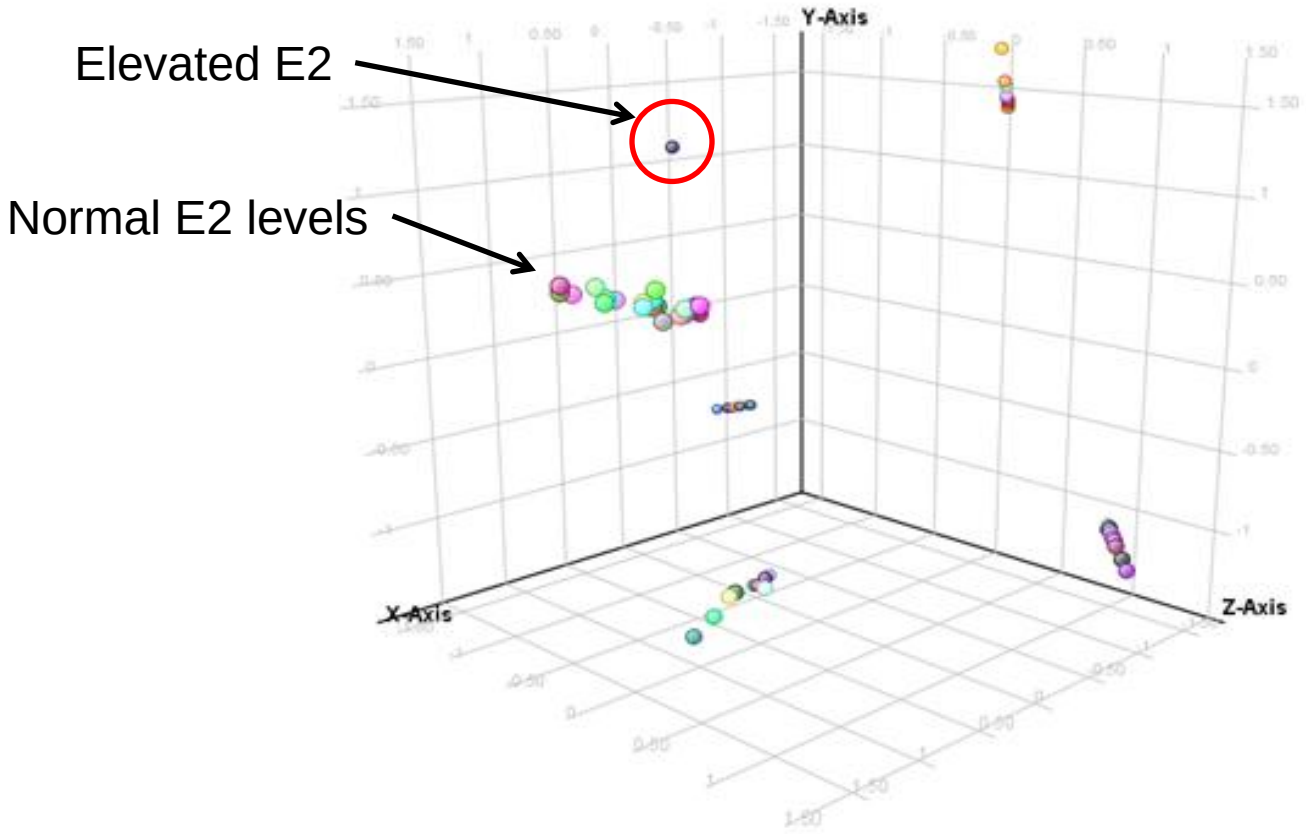
*Williard C. Application of GC/MS/MS in Monitoring Steroids as a Pharmacokinetic and Pharmacological Biomarker. Retrieved April 1, 2013 from, http://xtalks.com/xto525pharmanet-i3thankyou.ashx

Water sample chemotype alignment

E2 in water sources



Serum chemotype alignment



Summary of Study

Developed robust, accurate, precise and sensitive method to measure steroidal analogs

Important requirement to monitor the environment and the exogenous and endogenous exposome

Robust chemistry to modify steroidal analogs for NCI

Highly sensitive and robust analytical method with IDL on the order of < 1 pg/ml

- Waste water effluent and serum

Summary of Study

Using this model:

- Regional environmental identifies the presence of estrogens in the environment
  - Identifies outliers
- Exposome of inhabitants is measured
  - Identifies outliers
- Data is correlated with real health information
- Patients monitored for disease onset, progression, remission

Future: Develop predictive and preventative tools

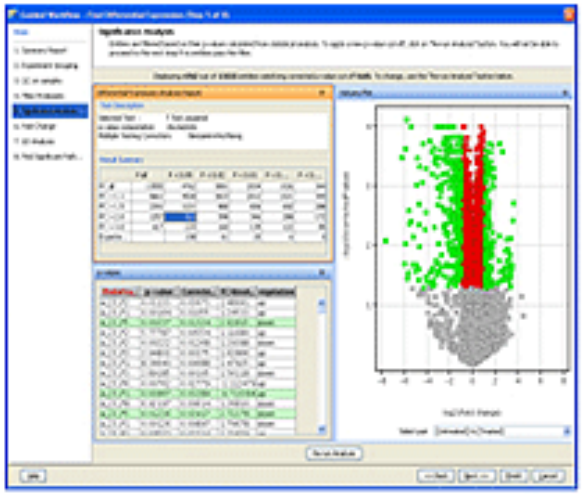
How can Agilent help?

Agilent has the total solution

- Broad industrial space: diagnostics, electronic manufacturing, LSCA…
  - All the omics tools under one roof
- Streamlined software suite: MassHunter, the GeneSpring family (MPP), pathway analysis, etc…
- Depth and breadth of expertise

Dako

An **Agilent Technologies** Company



Conclusion

Exposomics paradigm focuses on the environmental impact of exposure on disease

- Leverages GC/Q-TOF power in environmental, food safety *and* biological monitoring
- Cyclical exposomics
  - Agnostic non-targeted, targeted or hybrid panels focused on biologically relevant chemotypes
  - Multi disciplinary, multi-technique analyses

Software and Bioinformatics will be an integral component

- Multi-variant analysis
- Identify chemical fingerprints and biomarkers

Conclusion

Exposomics is:

- A nascent field ripe with opportunity
- Interdisciplinary / multi-technique

Exposomic markets:

- Epidemiology, pathology, “omics,” integrated biology, clinical chemistry, toxicology, environmental, food safety, pharma & biopharma, public health…

Exposomic assays:

- Estrogens and…
  - Urinary organic acids, amino acids, fatty acids, lipids, eicosanoids from n-3 fatty acids, emerging contaminates, endocrine disruptors, fluorotelomer alcohols, androgens, prostaglandins, acylglycines, α-keto acids, isotopomer flux, kynurenines…

Thank you.