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

Anthony Macherone, Ph.D.
Sr. Applications Chemist, Agilent Technologies
Visiting Scientist, the Johns Hopkins University School of Medicine
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
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

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

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

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

The exposome & exposomics

Radiation
Air and water pollution
Diet, Drugs, Stress, Infections
Behavior and lifestyle

Metabolism, Inflammation, Xenobiotics, Preexisting disease, Oxidative stress, Gut microflora

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

- **US: CDC, NIOSH, NIEHS, EPA**

- **UK Biobank**: Long-term follow-up of health (500,000 people ages 40 – 69)


- **May 24, 2013**: $4M Grant Awarded to Emory University, Georgia Tech to Create Exposome Center

---

**Agilent Technologies**
How do we measure the exposome

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*


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

<table>
<thead>
<tr>
<th>Time Segment</th>
<th>Compound</th>
<th>Precursor Ion</th>
<th>Product Ion</th>
<th>dwell</th>
<th>Collision Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E2</td>
<td>490.5</td>
<td>426.5</td>
<td>60</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>E1</td>
<td>464.4</td>
<td>400.4</td>
<td>60</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>EQ</td>
<td>462.4</td>
<td>398.4</td>
<td>60</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>EQ</td>
<td>462.4</td>
<td>370.4</td>
<td>60</td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>ASD</td>
<td>461.5</td>
<td>431.5</td>
<td>60</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>Testosterone</td>
<td>677.6</td>
<td>657.6</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Testosterone</td>
<td>677.6</td>
<td>627.6</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>E2</td>
<td>660.5</td>
<td>596.5</td>
<td>100</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>E3</td>
<td>870.6</td>
<td>806.6</td>
<td>150</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>E3</td>
<td>870.6</td>
<td>167.6</td>
<td>150</td>
<td>10</td>
</tr>
</tbody>
</table>
Column configuration

- MMI Pulsed Splitless (280°C)
- Pressure / Flow Controller
- Purged Ultimate Union
- 5m DB-17ht
- 15m DB-17ht
- 2.4 mL/min
- 1.25 mL/min
- 1.23 mL/min
- -6 mL/min
- 7000B
- NCI Mode
- SRM mode
- Source 150°C

Blue – Analysis
Red – Back Flush
Pre-Column BF Forward Flow

Inlet EPC

P₁

Split Vent

Coated Pre-Column

Aux EPC

P₂

Restrictor

Purged Union

Column

MSD

Agilent Technologies
Pre-Column Concurrent Backflush

- Inlet EPC
- Coated Pre-Column
- Purged Union
- Restrictor
- Aux EPC
- Column
- MSD

Split Vent

Pressures:
- $P_1$
- $P_2$
No BF

RT Shifting

First injection

7 replicate injections

Agilent Technologies
With BF
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)
- Equilin
- 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

E2 - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 0 QCs

\[ y = 143.639898 \times x - 20.525973 \]

\[ R^2 = 0.99804238 \]

0.5 pg/mL – 250 pg/mL
Estradiol, \( r^2 = 0.998 \)
Instrument Detection Limit (IDL): Water extracts

Determined by the equation:
IDL = (t_α)(%RSD_{area})(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 \text{ pg/ml})/100 = 0.13 \text{ pg/ml E2}
E2 linearity (serum)

Excellent Linearity Demonstrated Over 12 Hours

Analyte 1-10 Levels, 10 Levels Used, 10 Points Used, 0 QCs

\[ y = 0.0033 \times x + 0.0065 \]

\[ R^2 = 0.99994845 \]
E2 Replicate injections (serum): IDL = 0.41 pg/mL (recently reported at 0.12 pg/mL*)

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Concentration (pg/mL)</th>
<th>N</th>
<th>Response Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>EA-1-1</td>
<td>1.0</td>
<td>1</td>
<td>0.0071</td>
</tr>
<tr>
<td>EA-1-2</td>
<td>1.0</td>
<td>2</td>
<td>0.0087</td>
</tr>
<tr>
<td>EA-1-3</td>
<td>1.0</td>
<td>3</td>
<td>0.0086</td>
</tr>
<tr>
<td>EA-1-4</td>
<td>1.0</td>
<td>4</td>
<td>0.0087</td>
</tr>
<tr>
<td>EA-1-5</td>
<td>1.0</td>
<td>5</td>
<td>0.0078</td>
</tr>
<tr>
<td>EA-1-6</td>
<td>1.0</td>
<td>6</td>
<td>0.0076</td>
</tr>
<tr>
<td>EA-1-7</td>
<td>1.0</td>
<td>7</td>
<td>0.0047</td>
</tr>
<tr>
<td>EA-1-8</td>
<td>1.0</td>
<td>8</td>
<td>0.0085</td>
</tr>
<tr>
<td>EA-1-9</td>
<td>1.0</td>
<td>9</td>
<td>0.0078</td>
</tr>
<tr>
<td>EA-1-10</td>
<td>1.0</td>
<td>10</td>
<td>0.0079</td>
</tr>
</tbody>
</table>

Average 0.0077
Std. Dev. 0.0011
% RSD 14.69

Water sample chemotype alignment

E2 in water sources
Serum chemotype alignment

- Elevated E2
- Normal E2 levels
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
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 contaminants, endocrine disruptors, fluorotelomer alcohols, androgens, prostaglandins, acylglycines, α-keto acids, isotopomer flux, kynurenines…
Thank you.