High-Throughput Milk Oligosaccharide Analysis Using a Rapid Cartridge-Based Capillary Electrophoresis Instrument

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What is so beneficial about HMOs, if they are indigestible in the infant gut?
HMO Functions

Prebiotics for beneficial bacteria in the infant’s gut.

Infant protection by **inhibiting binding of pathogens** to epithelial surface

- Sialylated HMO may promote post-natal **brain development**.

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Newburg et al 2007
Human Milk Oligosaccharides Composition

Courtesy of Dr. Jasmine Davis
Importance of Secretor Status

- ABH antigens secreted in bodily fluids
- Indicator for being a secretor is α1-2 linked Fucose
- Typically around 20% of population are non-secretors

<table>
<thead>
<tr>
<th></th>
<th>Secretor</th>
<th>Non-secretor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower instances of diarrhea</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Promotion of a healthy microbiota</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>More protection against bacterial infections &amp; autoimmune diseases</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Higher amounts of total oligosaccharides</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>More resistant to norovirus, influenza, rhinovirus, and HIV-1</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Lower rate of several common digestive tract cancers</td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

Jasmine Davis, Sarah Totten

Nutrition Reviews, Vol. 67 (Suppl. 2), S183-S191.
Methods for detection and quantification of Oligosaccharides

**Separation**
- Liquid Chromatography
- Capillary Electrophoresis
- High pH Anion-Exchange Chromatography

**Detection**
- Ultraviolet detection
- Fluorescence Detection
- Nuclear Magnetic Resonance
- Mass Spectrometry

Gly-Q
1809: Ferdinand Frederic Reuss suspended clay particles in an aqueous solution to migrate under the influence of an electric field

1950: Gel electrophoresis

1981: James Jorgenson demonstrated that the use of capillaries overcome many of the heating issues and stabilizing media are no longer necessary

1989: Developed the first commercial instrument

Currently capillary electrophoresis is used for analysis of Nucleic acids, Proteins, Metabolites, Pharmaceuticals and Carbohydrates

Why develop methods to analyze oligosaccharides using Gly-Q?
Gly-Q: Capillary electrophoresis in a cartridge

- Anionic Label
- OS Sample

- Separation occurs less than 2 minutes
Gly-Q Manager Software

**Gly-Q: Data Analysis**

- DP2 and DP15 standards co-injected with samples for alignment
- GU ladder injected in sequence to automatically generate GU values

**Alignment of peaks**

- Peak assignment using GU values
## Gly-Q: Characteristics

<table>
<thead>
<tr>
<th>Features</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throughput</td>
<td>2 x 96 well plates per day</td>
</tr>
<tr>
<td>Analysis time per sample</td>
<td>&lt;2 minutes per sample</td>
</tr>
<tr>
<td>Injection</td>
<td>Electrokinetic (Virtually no sample consumption like CE)</td>
</tr>
<tr>
<td>Detection</td>
<td>fluorescence detection</td>
</tr>
<tr>
<td>Ease of use</td>
<td>Click-in capillary, 96-well plate-compatible</td>
</tr>
<tr>
<td>Instrument Footprint</td>
<td>15” x 16” x 12” (L x H x W) 38cm x 40cm x 30cm</td>
</tr>
</tbody>
</table>
Profiling Milk Oligosaccharides
Human Milk Oligosaccharide Analysis Workflow

1. Milk
   - Protein & lipid removal

2. Milk oligosaccharides
   - Label
     - APTS label

3. Labeled oligosaccharides + dye
   - Cleanup
     - Centrifugation
     - Ethanol precipitation
     - Solvent dry down

4. Labeled Oligos
   - Sample analysis
     - HILIC

5. Data
   - 2 minute Gly-Q experiment
Gly-Q Analysis of HMOs
HMO Standards Analyzed with Gly-Q
### GU Library with Oligosaccharide Standards

<table>
<thead>
<tr>
<th>Oligosaccharide</th>
<th>GU Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6’SLO</td>
<td>2.57</td>
</tr>
<tr>
<td>6’SLON</td>
<td>2.68</td>
</tr>
<tr>
<td>3’SLO</td>
<td>2.95</td>
</tr>
<tr>
<td>3’SLON</td>
<td>3.11</td>
</tr>
<tr>
<td>DSLNT</td>
<td>3.73</td>
</tr>
<tr>
<td>3FL</td>
<td>3.96</td>
</tr>
<tr>
<td>2’FL</td>
<td>4.01</td>
</tr>
<tr>
<td>LSTc</td>
<td>4.03</td>
</tr>
<tr>
<td>LSTa</td>
<td>4.21</td>
</tr>
<tr>
<td>LSTb</td>
<td>4.42</td>
</tr>
<tr>
<td>LDFT</td>
<td>4.61</td>
</tr>
<tr>
<td>LNnT</td>
<td>4.84</td>
</tr>
<tr>
<td>LNT</td>
<td>5.11</td>
</tr>
<tr>
<td>LNFP I</td>
<td>5.90</td>
</tr>
<tr>
<td>LNDFH I</td>
<td>6.01</td>
</tr>
<tr>
<td>LNFP V</td>
<td>6.07</td>
</tr>
<tr>
<td>LNFP III</td>
<td>6.08</td>
</tr>
<tr>
<td>LNDFH II</td>
<td>6.36</td>
</tr>
<tr>
<td>LNNH</td>
<td>6.49</td>
</tr>
<tr>
<td>MFLNH III</td>
<td>7.25</td>
</tr>
</tbody>
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- GU library is used to automatically label the oligosaccharide peaks
Secretor Status
Profiles of Secretor Mother’s Milk Oligosaccharides

Signal (%)

Time (min.)

Secretor 1

Secretor 2

Secretor 3
Profiles of Non-Secretor Mother’s Milk Oligosaccharides

Signal (%)

Time (min.)

Non-Secretor 1

Non-Secretor 2

Non-Secretor 3

Lactose

3FL and 2'FL

LNFP III + LNFP V

LNFP I

LNDFH I

6'SL

6'SLN

3'SL

DSLNT

LNT

LDFT

LSTb

LNNt
Identification of Secretor Status

Nano-HPLC-Chip/TOF MS data of Secretor Status

Secretor

Non-secretor
Quantification of Oligosaccharide in Bovine Milk
Bovine Milk

Less complex than HMO
1-1.5 g/L OS in colostrum, 0.1-0.2 g/L OS in mature milk

Diverse dairy products

Sample A
Whey permeate
Still contains OS, Lactose, minerals

Sample B
Bovine milk OS concentrate

Industrial process
Bovine Milk Oligosaccharide Analysis Workflow

- **Milk Permeate**
  - Internal Std: DP7

- **Label**
  - Labeled oligosaccharides + dye

- **Cleanup**
  - Labeled Oligos
  - *APTS label*  
  - *HILIC*

- **Sample analysis**
  - 2 minute Gly-Q experiment

- **Data**
Electropherogram of Sample A (Whey Permeate)
Electropherogram of Sample B (OS Concentrate)
Electropherogram of Sample A (Whey Permeate)

Sample A
Whey permeate

Sample B
Bovine milk OS concentrate

Industrial process

Electropherogram of Sample A (Whey Permeate):
- 6SL: 0%
- 3SL: 2%
- Lac: 98%

Electropherogram of Sample B:
- 6SL: 5%
- 6SLN: 9%
- 3SLN: 12%
- LSTa: 2%
- LNnT: 1%
- 2FL, 3FL, LSTc: 2%
- Lac: 0%
Comparability of Gly-Q to Dionex Method

Sample A
Whey permeate

Sample B
Bovine milk OS concentrate

Industrial process

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Industrial process
Conclusion

- A rapid and sensitive methods were developed for quantitative and qualitative analyses of oligosaccharides using Gly-Q.

- Sample preparation is simple, rapid and automatable

- Fast separation (< 2 min)

- Automated data analysis

- Suited for large number of sample analyses
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