Sugar Extraction Study
Round 3!

Initial Study 2010-11, Extractions by each Lab
Second Study 2011-12, All Extractions by one Lab
Third Study 2013, No Extractions, All Pure Standards

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Initial Study 2010-11

• Compared multiple feeds with each lab providing their internal sample preparation methodology.

• Large standard deviations existed in reported data across multiple feed samples.
2nd Study 2011-12, Extraction Study

- Observe agreement between labs from single source extractions

- Compare 50/50 ACN – water vs. Water only extraction.
3rd Study 2013-14
Pure Standards Only

- Discover Best General Precision for Sugar Analysis Under Ideal Conditions and Concentrations
- Identify Best Available Technologies (if possible!)
Experimental Design

Fructose, Galactose, Glucose, Lactose, Maltose and Sucrose Standards.

• 10 Labs x 6 Sugars x 2 Concentrations
• Each Lab gets 4 Samples, 2 A and 2 B
  • A contains each Sugar at 1% Solution
  • B contains each Sugar at 0.1% Solution
• On the first day run 1 A and 1 B both in duplicate under strict repeatability conditions.
• On another day run the remaining A and B samples in duplicate under strict repeatability conditions.
Experimental Design

10 Labs Get Samples for This Scenario

Ax2 1%

Day 1
A1
A2

Day 2
A1
A2

Bx2 0.1%

Day 1
B1
B2

Day 2
B1
B2

10 Labs x 2 Days x 2 Concentrations x 6 Sugars x 2 Duplicates = 480 Analytical Measurements
Sugars 1 % Raw Data (Fr, Ga, Gl, La, Ma, Su)

mg/100mL

LAB

Rep a
Rep b
Mean
Observed Mean Sugar Concentrations
Compare with Expected (1 %)

Standard Error ~ 0.01%
Observed Mean Sugar Concentrations
Compare with Expected (0.1 %)

- Fructose
- Galactose
- Glucose
- Lactose
- Maltose
- Sucrose

Standard Error ~ 0.0015%
## Which Technology is Better?

### Within Technology Precision at 1% Sugar

<table>
<thead>
<tr>
<th>Lab</th>
<th>Technology</th>
<th>Mean</th>
<th>Strict %rsd</th>
<th>Day %rsd</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>HPLC (ELSD)</td>
<td>1.00</td>
<td>3.5%</td>
<td>5.4%</td>
</tr>
<tr>
<td>C-1</td>
<td>GC (FID)</td>
<td>1.04</td>
<td>1.8%</td>
<td>3.8%</td>
</tr>
<tr>
<td>C-2</td>
<td>HPAEC (PAD)</td>
<td>0.97</td>
<td>1.0%</td>
<td>4.0%</td>
</tr>
<tr>
<td>D</td>
<td>HPLC (ELSD)</td>
<td>1.29</td>
<td>3.0%</td>
<td>6.2%</td>
</tr>
<tr>
<td>E</td>
<td>UPLC (RI)</td>
<td>0.91</td>
<td>1.1%</td>
<td>0.8%</td>
</tr>
<tr>
<td>F</td>
<td>HPLC (RI)</td>
<td>0.98</td>
<td>2.1%</td>
<td>2.2%</td>
</tr>
<tr>
<td>G</td>
<td>HPLC (RI)</td>
<td>1.07</td>
<td>1.2%</td>
<td>1.6%</td>
</tr>
<tr>
<td>H</td>
<td>HPLC (Post Column FLD)</td>
<td>1.05</td>
<td>1.4%</td>
<td>1.6%</td>
</tr>
<tr>
<td>I</td>
<td>IC (EC detection)</td>
<td>1.06</td>
<td>0.4%</td>
<td>0.8%</td>
</tr>
<tr>
<td>J</td>
<td>IC (EC detection)</td>
<td>1.10</td>
<td>4.7%</td>
<td>2.2%</td>
</tr>
</tbody>
</table>
### Within Technology Precision at 0.1% Sugar

<table>
<thead>
<tr>
<th>Lab</th>
<th>Technology</th>
<th>Mean</th>
<th>Strict %rsd</th>
<th>Day %rsd</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>HPLC (ELSD)</td>
<td>0.100</td>
<td>2.2%</td>
<td>4.4%</td>
</tr>
<tr>
<td>C-1</td>
<td>GC (FID)</td>
<td>0.089</td>
<td>0.0%</td>
<td>3.0%</td>
</tr>
<tr>
<td>C-2</td>
<td>HPAEC (PAD)</td>
<td>0.098</td>
<td>2.4%</td>
<td>4.0%</td>
</tr>
<tr>
<td>D</td>
<td>HPLC (ELSD)</td>
<td>0.104</td>
<td>0.8%</td>
<td>3.4%</td>
</tr>
<tr>
<td>E</td>
<td>UPLC (RI)</td>
<td>0.092</td>
<td>6.4%</td>
<td>3.2%</td>
</tr>
<tr>
<td>F</td>
<td>HPLC (RI)</td>
<td>0.109</td>
<td>19.0%</td>
<td>10.1%</td>
</tr>
<tr>
<td>G</td>
<td>HPLC (RI)</td>
<td>0.104</td>
<td>6.2%</td>
<td>4.6%</td>
</tr>
<tr>
<td>H</td>
<td>HPLC (Post Column FLD)</td>
<td>0.105</td>
<td>1.5%</td>
<td>4.0%</td>
</tr>
<tr>
<td>I</td>
<td>IC (EC detection)</td>
<td>0.101</td>
<td>0.2%</td>
<td>1.4%</td>
</tr>
<tr>
<td>J</td>
<td>IC (EC detection)</td>
<td>0.112</td>
<td>1.3%</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

**Which Technology is Better?**
ANOVA: Which Technology is Better?

*Each Technology represented by only one lab
ANOVA: Which Technology is Better?

*Sugars (%)

*Each Technology represented by only one lab
General Precision for Sugars Analysis Under Ideal Conditions

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Strict %rsd</th>
<th>Day %rsd</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Sugars</td>
<td>1.05</td>
<td>2.5%</td>
<td>3.7%</td>
<td>10.8%</td>
</tr>
<tr>
<td>0.1% Sugars</td>
<td>0.101</td>
<td>6.8%</td>
<td>4.7%</td>
<td>10.1%</td>
</tr>
</tbody>
</table>

Within

Between
Method Needs Statement

LOQ = 100 mg/100mL (0.1 %)
Repeatability (CVR) at 2 x LOQ ≤ 5%

LOQ defined as the lowest concentration at or above which the analyte can not only be reliably detected but at which some predefined goals for bias and precision are met.

Table 1. Recommended Method Performance Characteristics:

<table>
<thead>
<tr>
<th>Each compound</th>
<th>Method LOQ, %</th>
<th>Operational concentration range, %</th>
<th>Accuracy at LOQ</th>
<th>Accuracy at midrange</th>
<th>Repeatability (CVR) at Midrange</th>
<th>Repeatability (CVR) at 2xLOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Each compound</td>
<td>0.1%</td>
<td>0.1% – 100%</td>
<td>90% - 108%</td>
<td>92% - 105%</td>
<td>= or &lt; 4%</td>
<td>= or &lt; 5%</td>
</tr>
</tbody>
</table>
What’s Next?
Efficacy of Extraction Procedures
Can we retain this Precision?