

Optimizing Calibration Techniques for Enhanced Fermentation Analysis in Biofuel Production

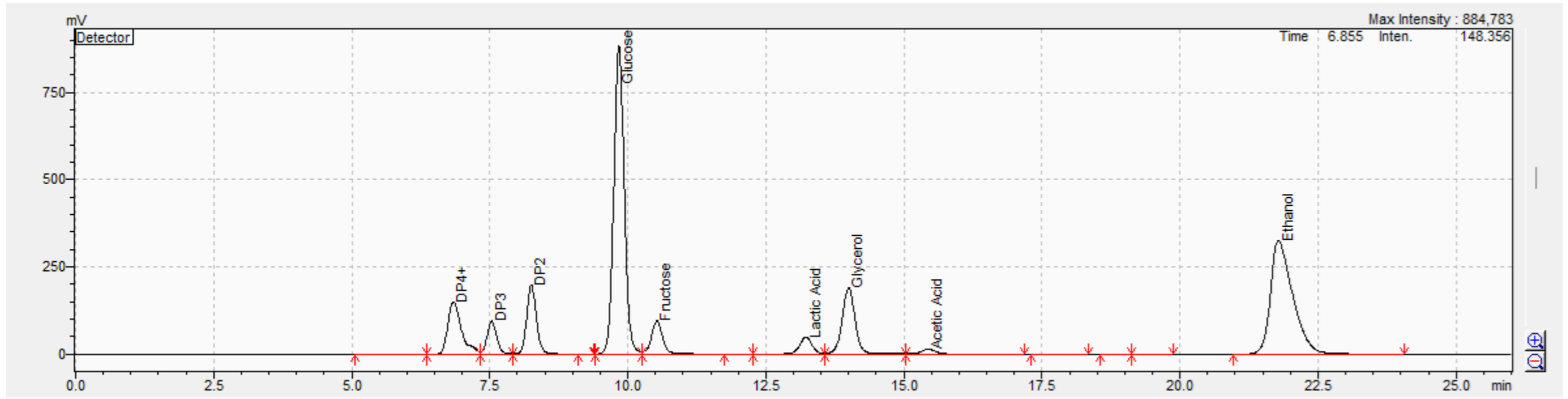
Charlette Hasty



CTE GLOBAL

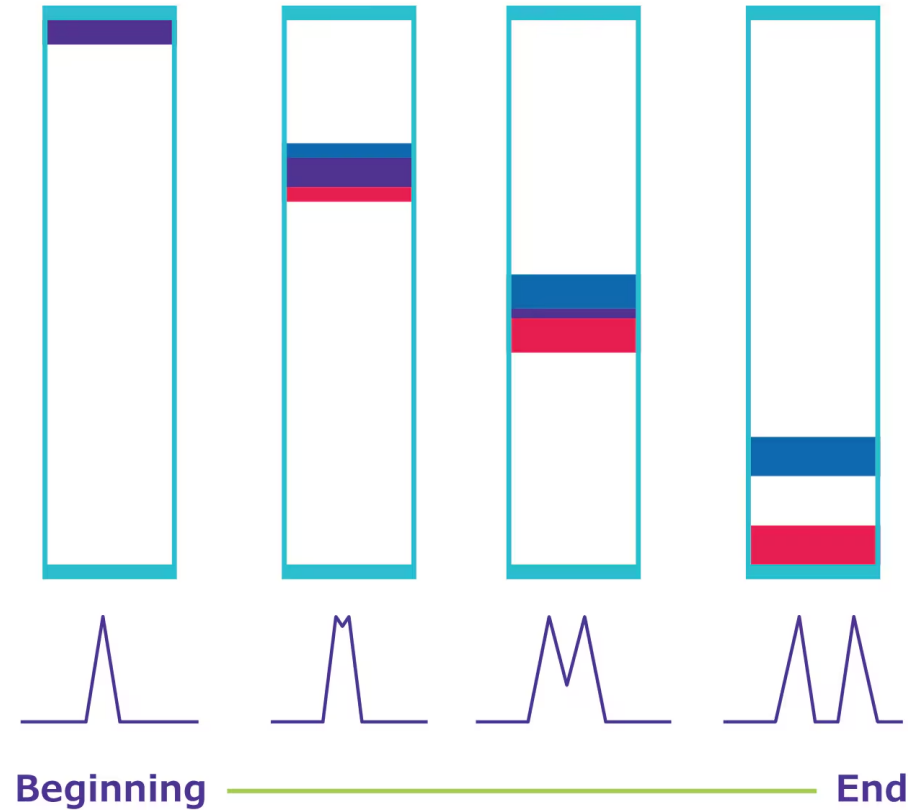
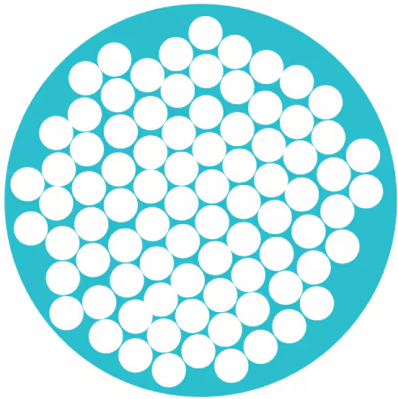
High Performance Liquid Chromatography

Liquid chromatography is a separation technique for nonvolatile liquid mixtures
High-performance liquid chromatography (HPLC) is the modern form of LC and is able to separate complex mixtures in a short amount of time

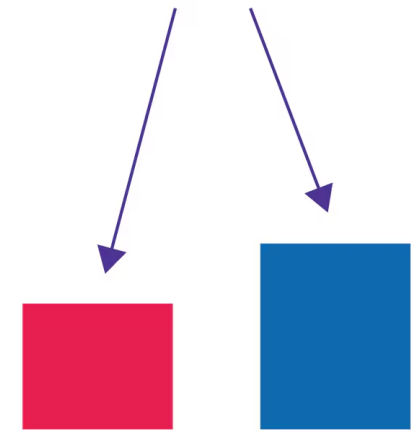


How does the HPLC Work?

Particles in the HPLC columns



Resolved Components



Why is HPLC Data So Important?



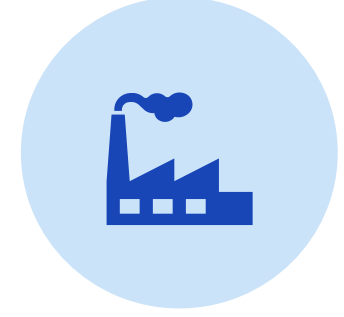
SUGAR CONVERSION
THROUGHOUT
FERMENTATION



ENZYME
OPTIMIZATION



YEAST HEALTH



EFFICIENCY
EVALUATION
(ETHANOL
PRODUCTION)



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Objectives



Compare performance between shorter (150 mm) and longer (300 mm) HPLC columns used in the Ethanol Industry.



Investigate the precision of 6-point, 3-point, and 1-point calibration curves when used with both column lengths.



Review a case study examining and addressing challenges in HPLC calibration.

Long vs. Short Column

Long Columns (300mm):

- Increase Analysis Time (~22-23 minutes)
- Improved sensitivity
- Improved resolution

Short Columns (150 mm):

- Faster Analysis Time (~11-12 minutes)
- Decreased sensitivity
- Decreased resolution



Factors Affecting Resolution

Retention Factor

- Also known as “capacity factor”
- Describes how well the analyte is retained on the column
- Dependent upon solvent strength, not column length or flow rate

Selectivity

- Ability to distinguish between different compounds
- Depends on column chemistry and mobile phase

Efficiency

- Related to theoretical plates
- Describes the distribution of the analyte’s particles; how narrow is the peak
- Depends on flow rate, column length, and particle size

$$R_s = \frac{k}{k+1} \times \frac{a-1}{a} \times \frac{\sqrt{N}}{4}$$

Retention Selectivity Efficiency

Column Comparison: Conditions

Phenomenex Rezex ROA- Organic Acid Column

300 X 7.8 mm

150 X 7.8 mm

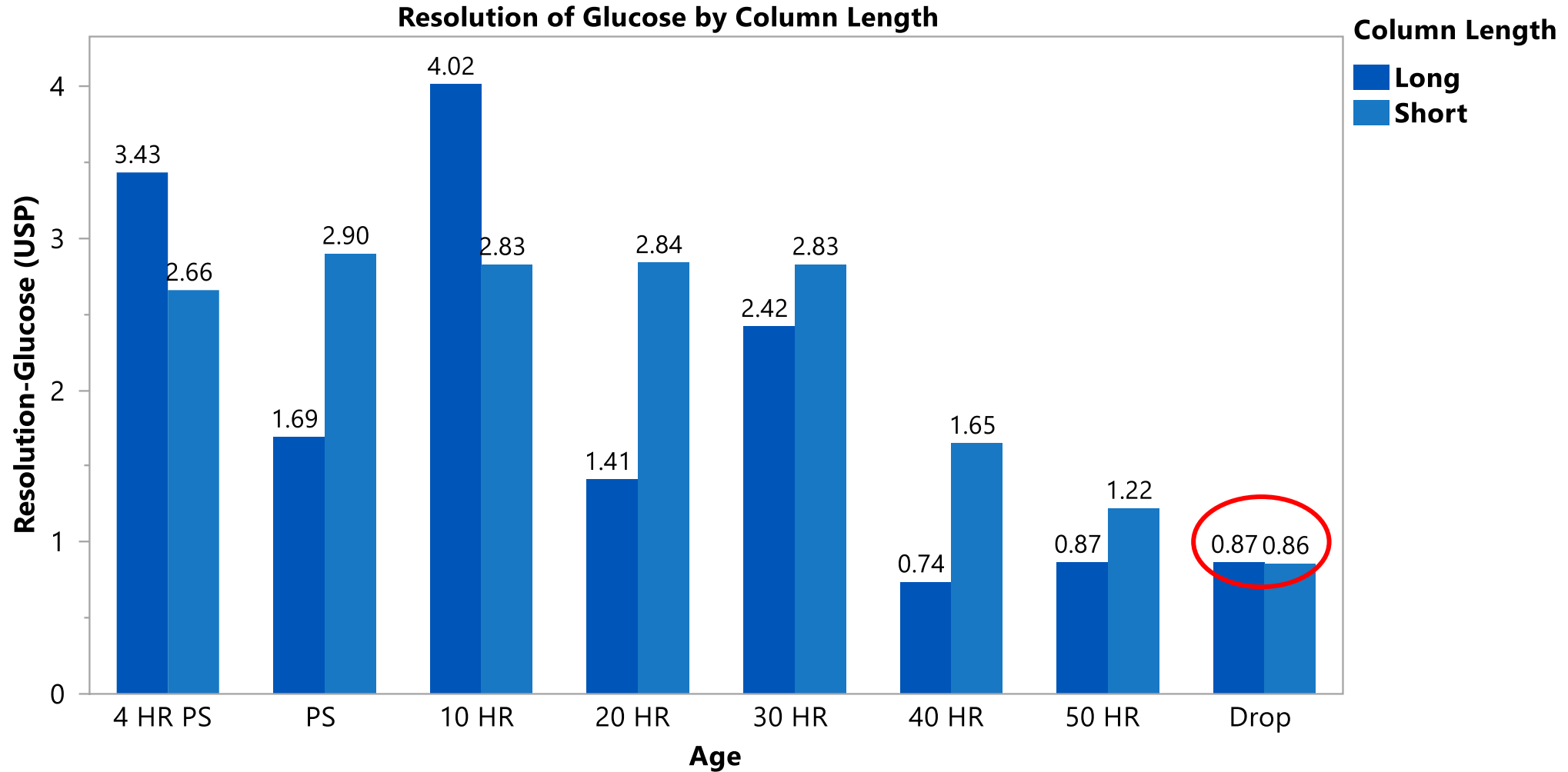
Mobile Phase: 0.005 N H₂SO₄

Flow Rate: 0.600 ml/min

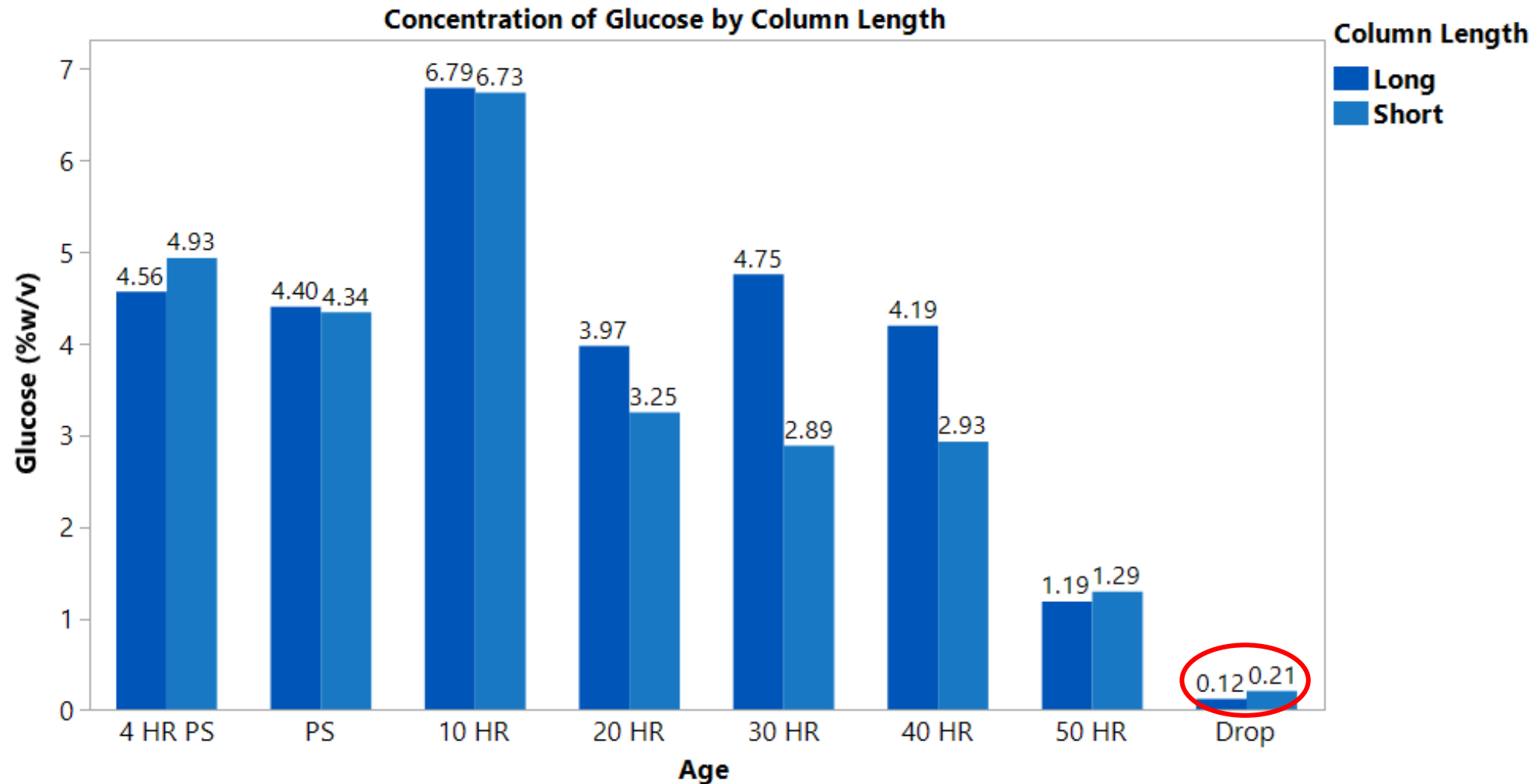
Oven Temp: 60 °C

(7)-Plant fermentation samples

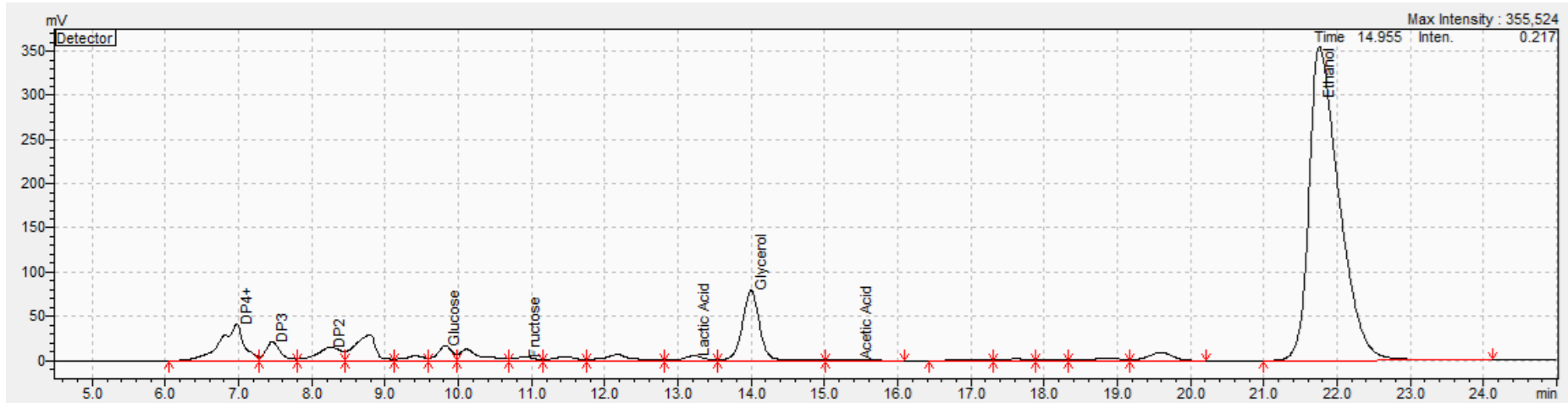
Resolution Results



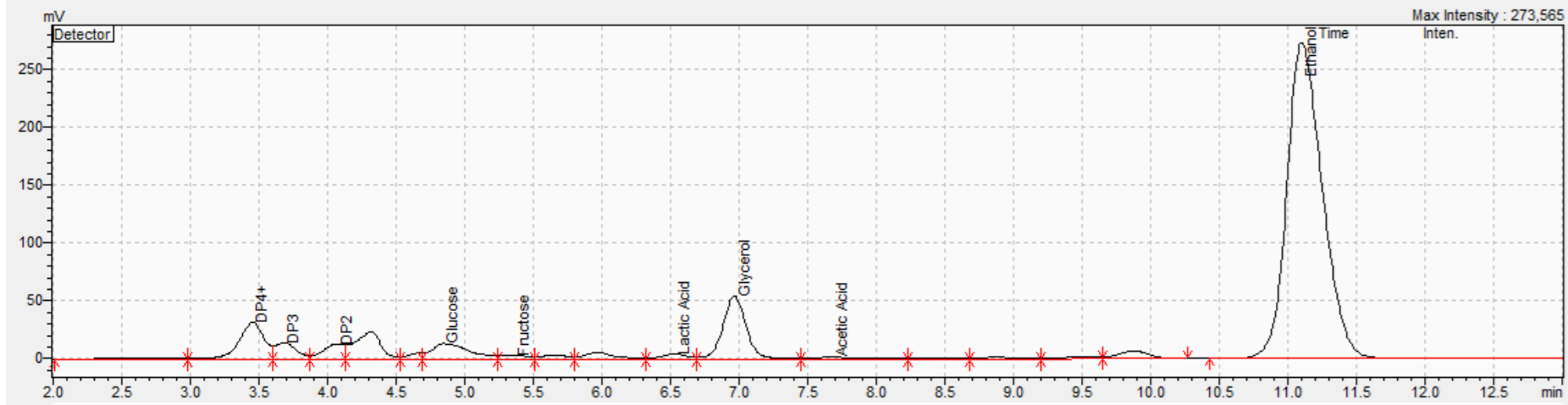
Concentration Results



Visual Comparisons- Drop Samples

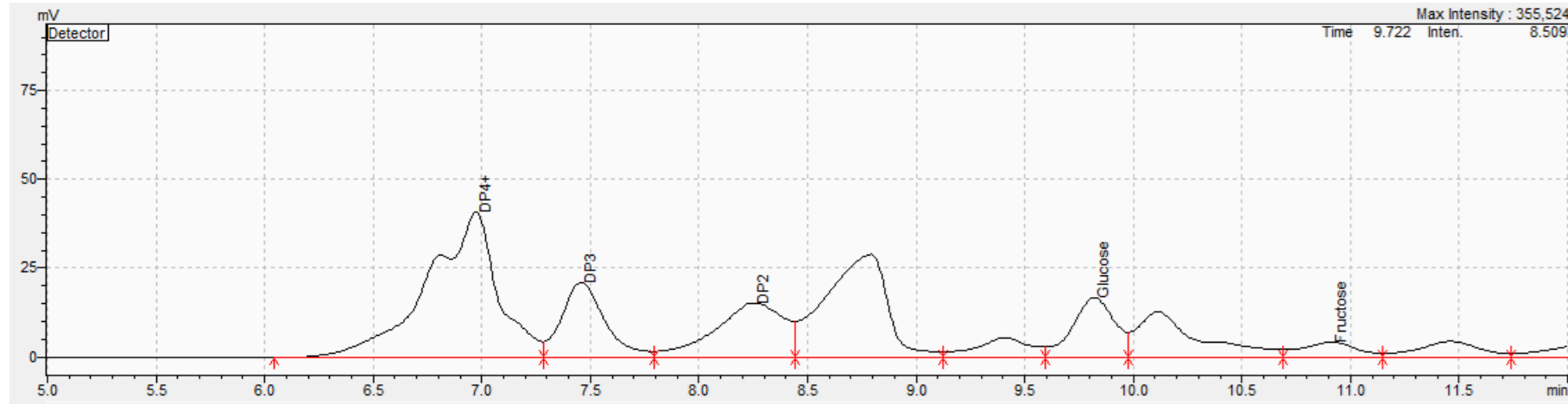


300mm Column

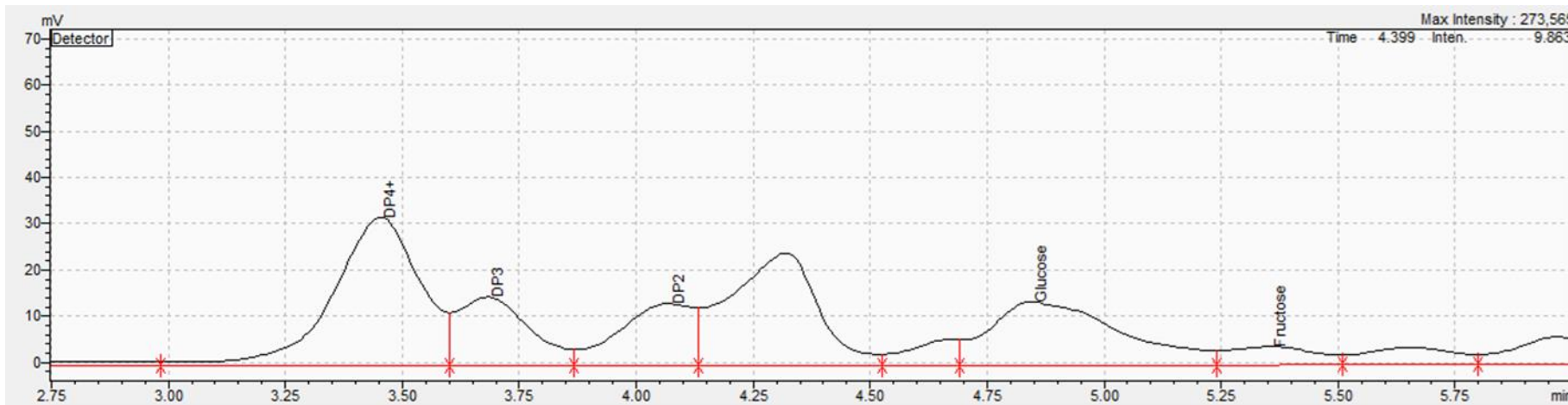


150 mm Column

Visual Comparisons- Drop Samples

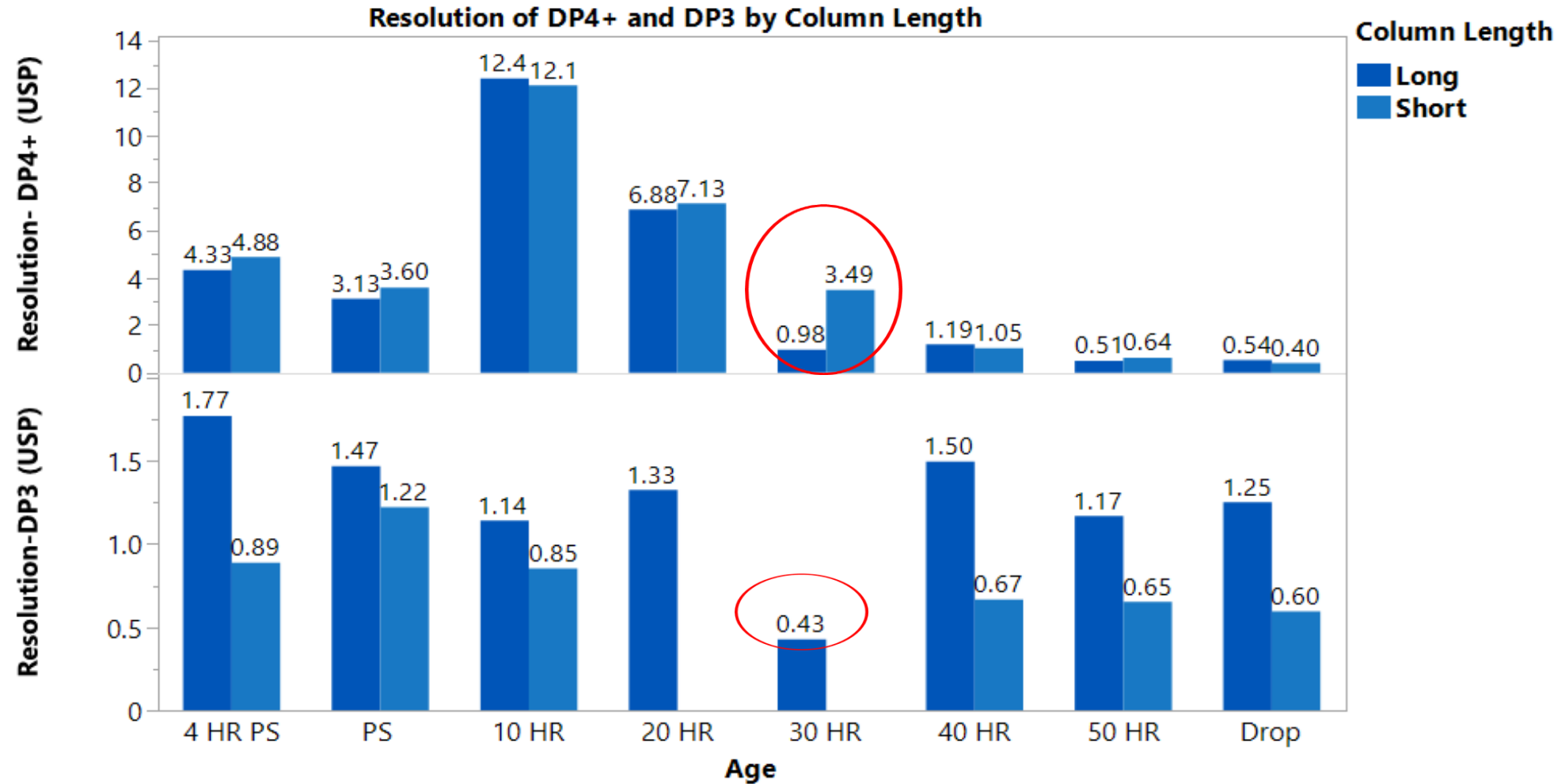


300mm Column

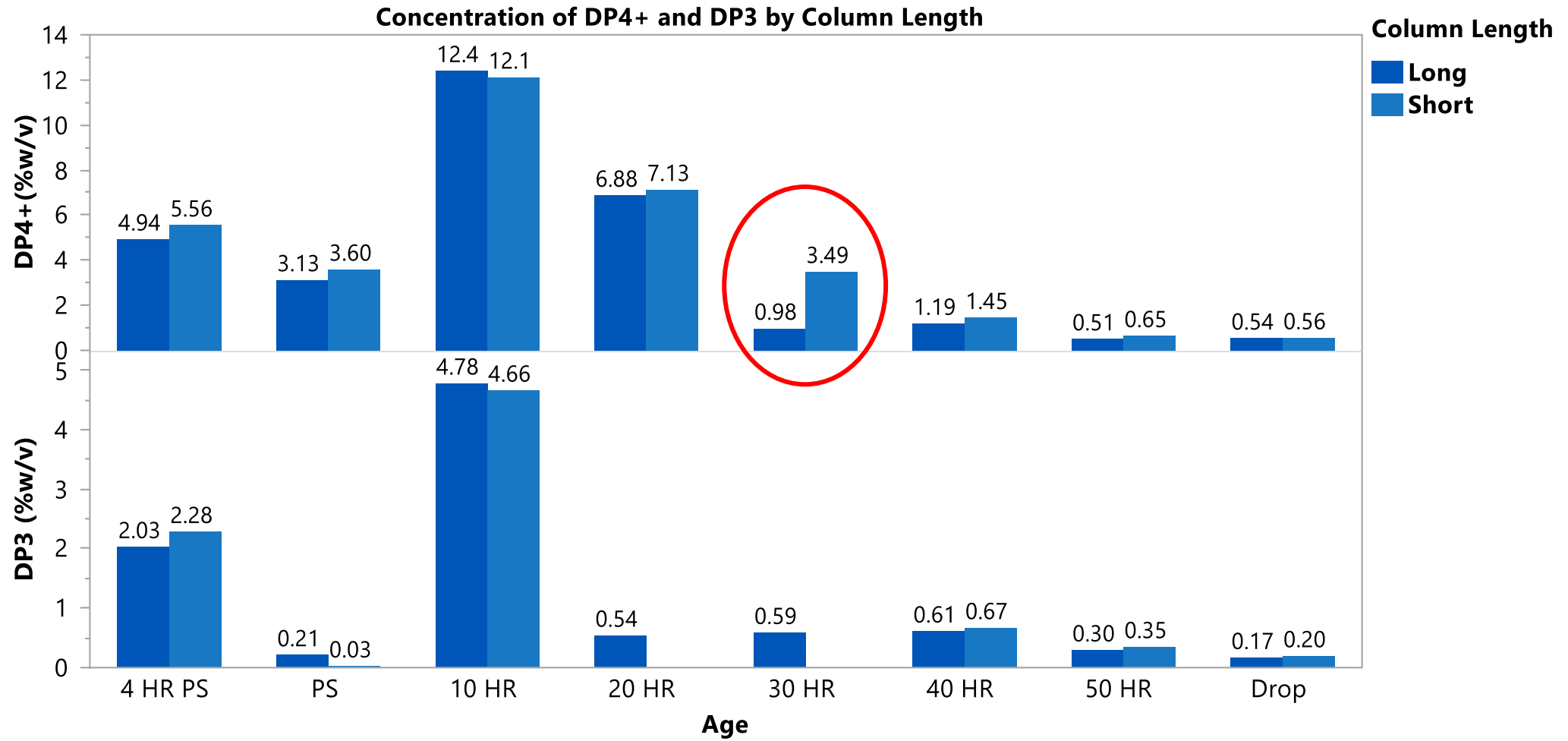


150 mm Column

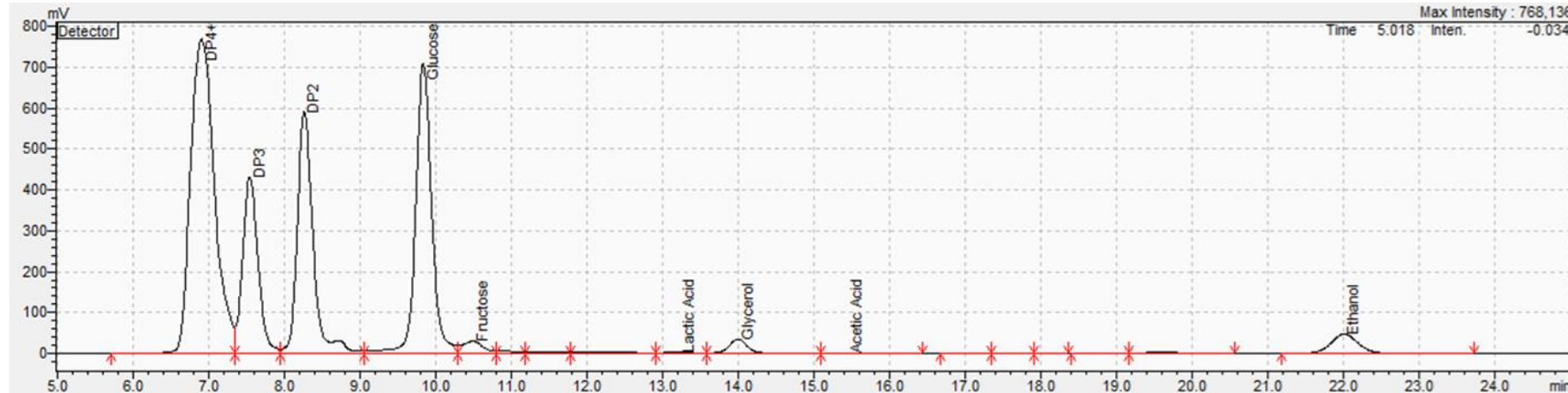
Resolution Results



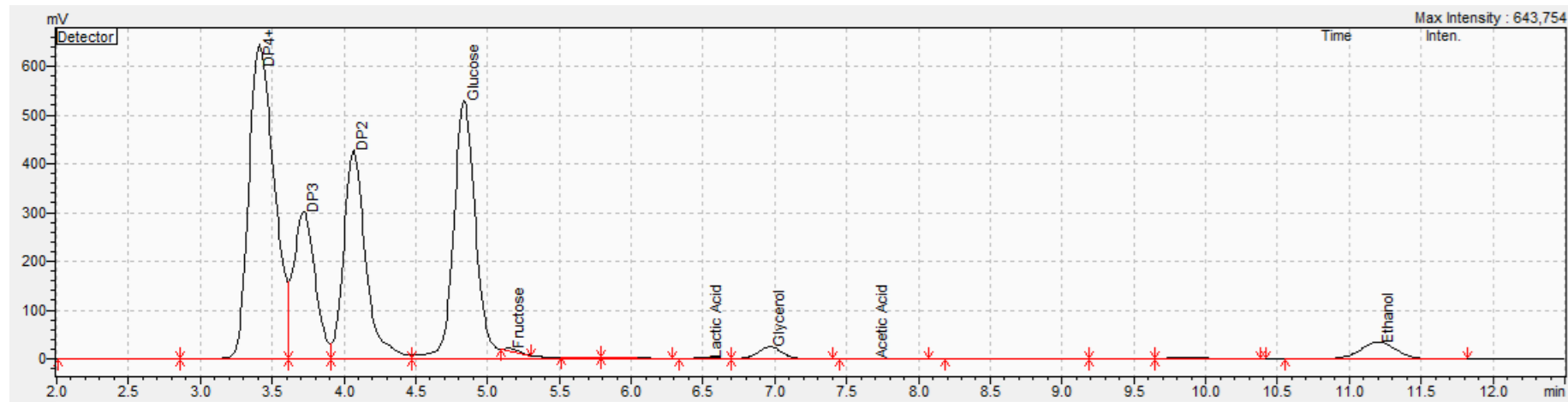
Concentration Results



Visual Comparisons- 10-Hour Sample

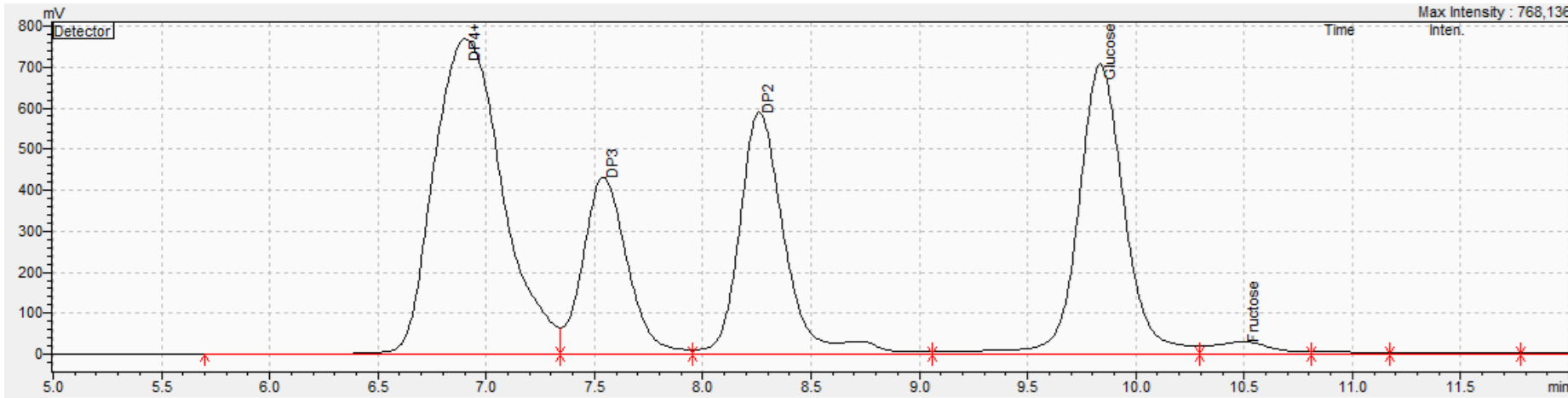


300mm Column

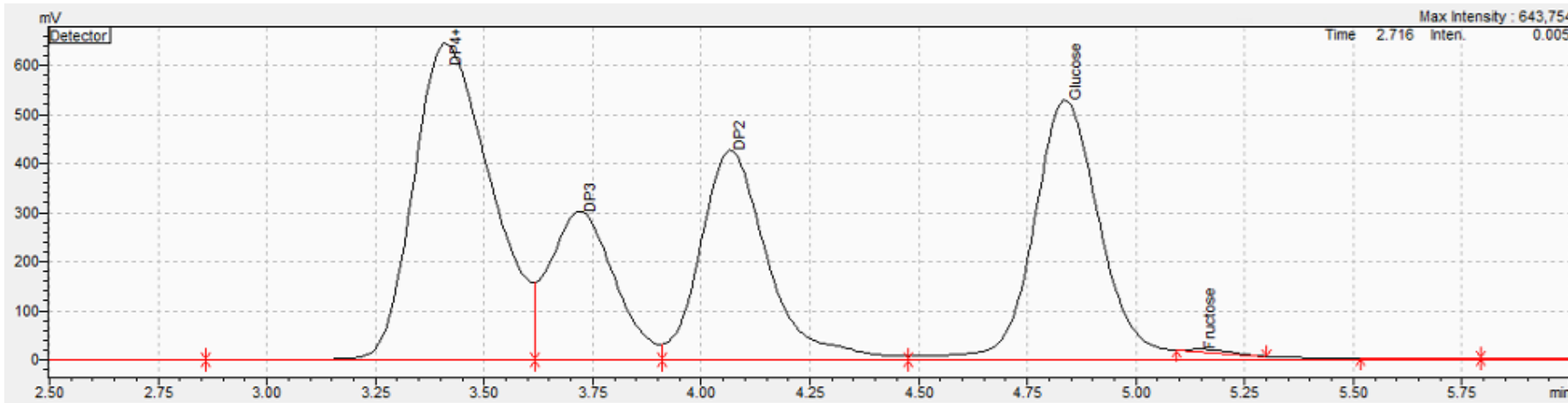


150 mm Column

Visual Comparisons- 10-Hour Sample

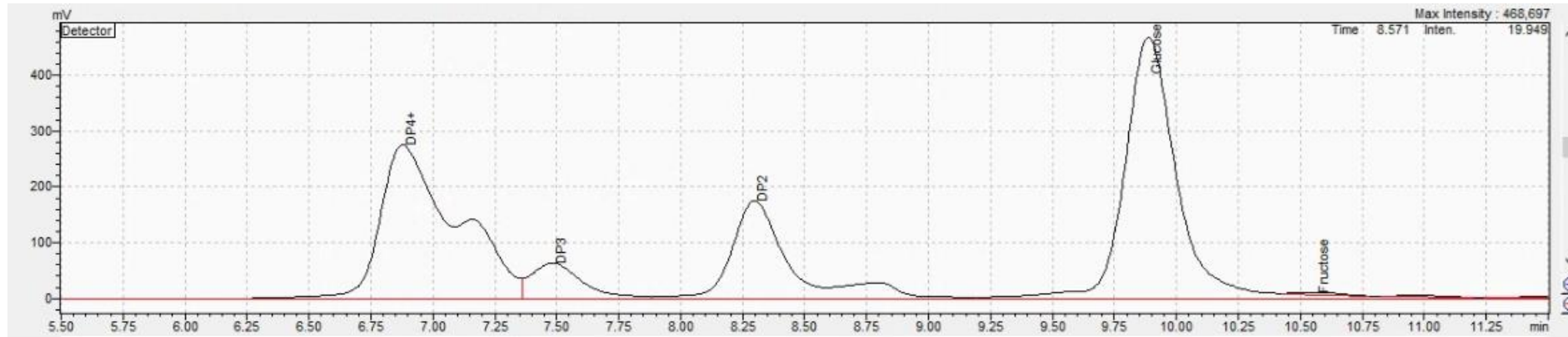


300mm Column

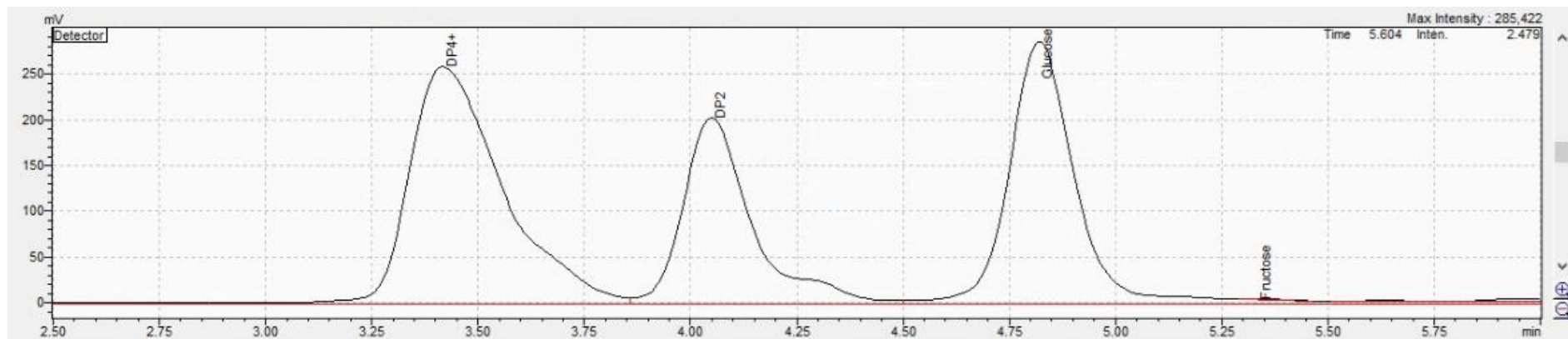


150 mm Column

Visual Comparisons- 30 Hour Sample

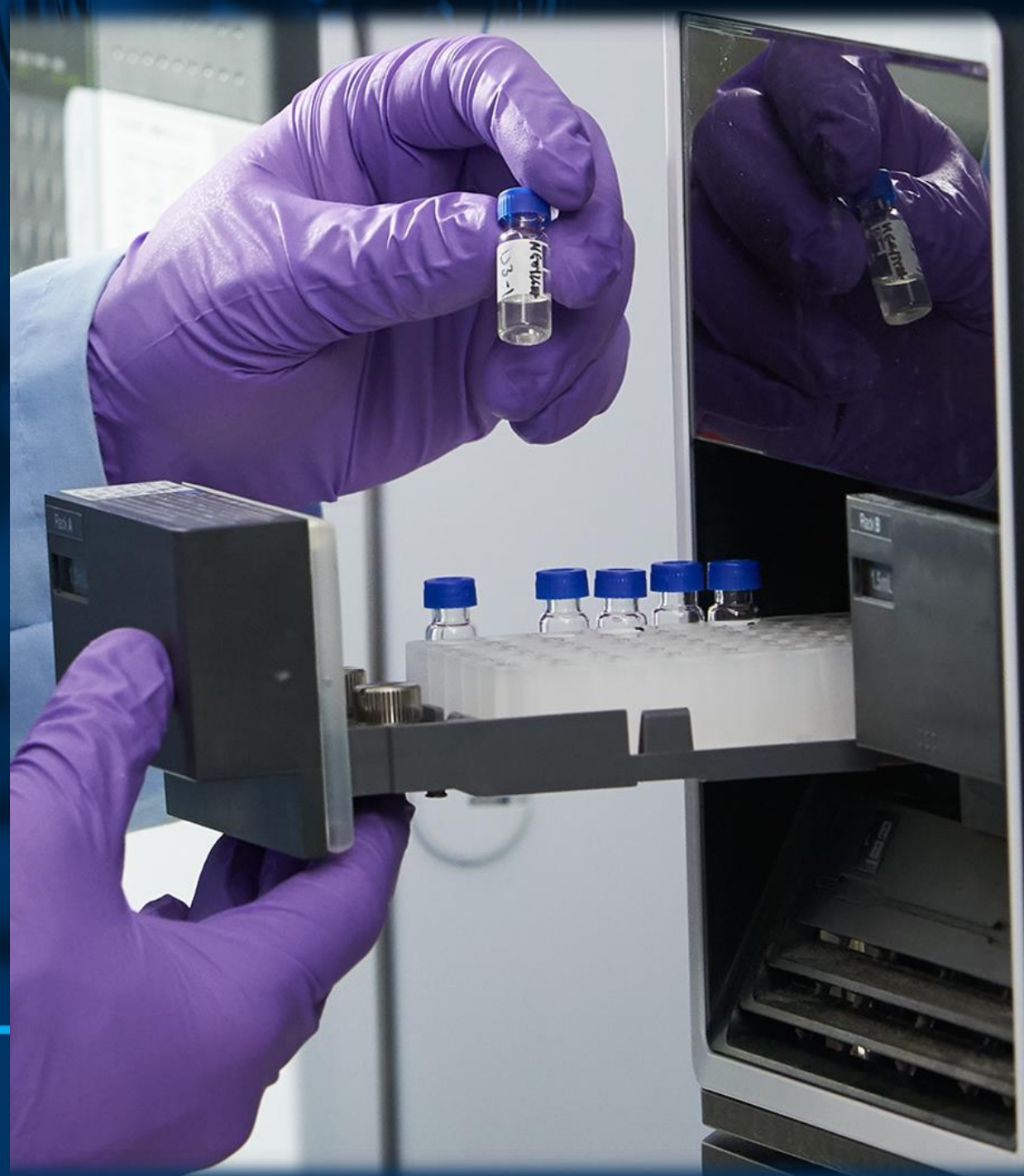


300mm Column



150 mm Column

From Column Length to
Calibration Accuracy:
Evaluating Concentrations with
Different Calibration Curves



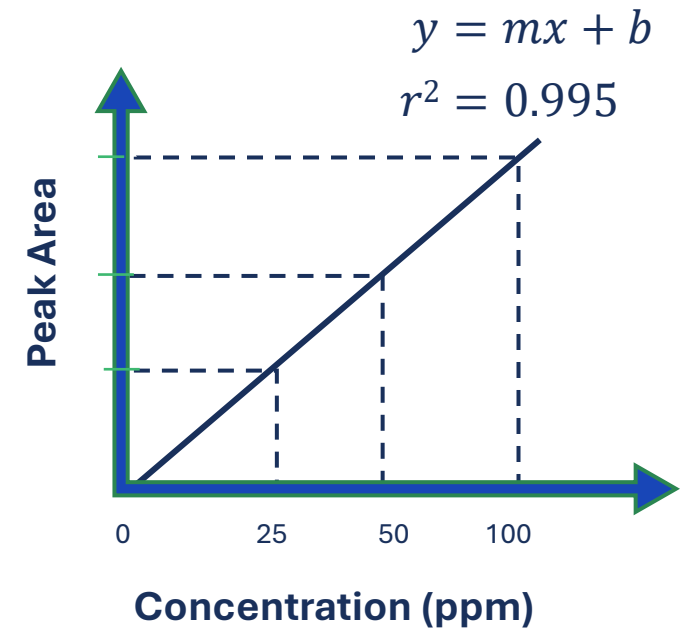
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How are Concentrations obtained?

A calibration curve is a plot that shows the relationship between known concentrations of a substance (analyte) and the detector's response (e.g., peak area or height) in HPLC.

How it's Constructed:

- Known standard concentrations are injected into the HPLC.
- The detector response (e.g., peak area) is plotted against the analyte concentration.
- The curve should be linear, following the equation $y=mx+b$, where:
 - y = detector response
 - x = concentration
 - m = slope (sensitivity)
 - b = intercept (baseline response)



More Points, More Accuracy: The Power of Calibration Curves



Quantification: Calibration curves allow us to determine the concentration of unknown samples by comparing their detector response to the known standards.



Ensures Accuracy: Calibration curves verify the accuracy of the HPLC system by showing the relationship between analyte concentration and response across a defined range.



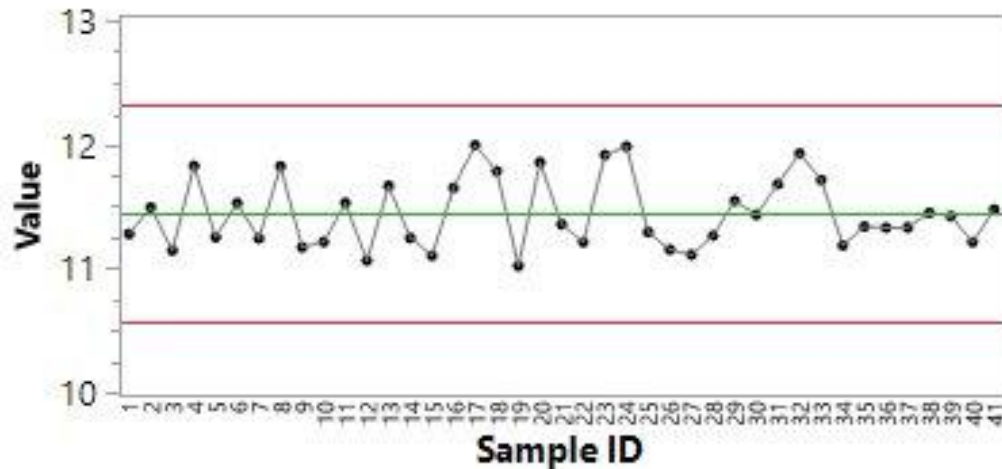
Linearity Check: The curve helps confirm that the detector's response is linear over the range of interest, which is crucial for precise measurements.



System Validation: A well-constructed calibration curve ensures that the system is functioning properly, preventing inaccurate or biased results.

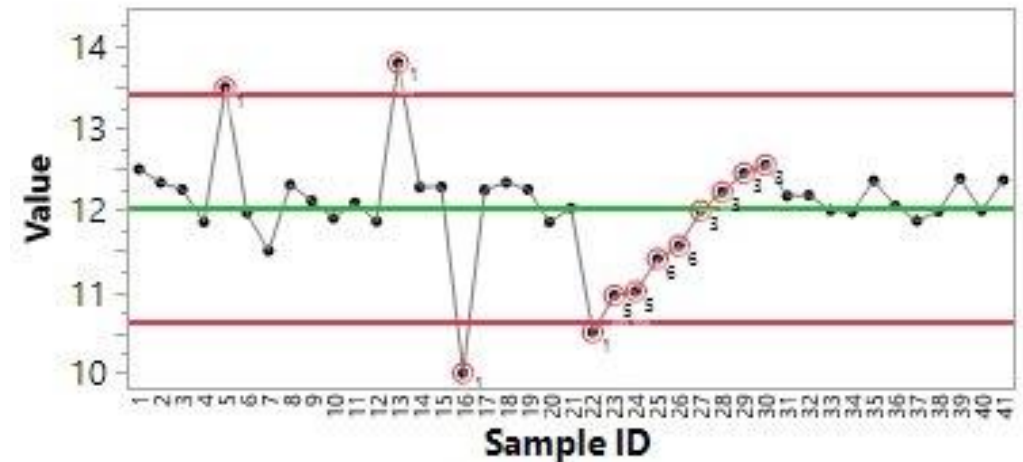
The Importance of Validation Standards

- Ensures Accuracy and Reliability of Analytical Results
- Increases the confidence in our HPLC results for our sample sets
- Minimizes Errors and Reduces Retesting Costs
- Validation standard concentrations are plotted on a control chart and monitored over time



This process is stable

Data is distributed randomly and does not violate any of the control chart tests



This process is not stable

Data is erratic and violates several control chart tests

Why Use % Error to Evaluate Calibration Curves?



Quantifies Deviation: % error measures how much values deviate from the 6-point calibration curve, providing a clear indicator of calibration accuracy.



Assesses Precision: A lower % error means higher precision, ensuring the calibration curve consistently predicts concentrations accurately.

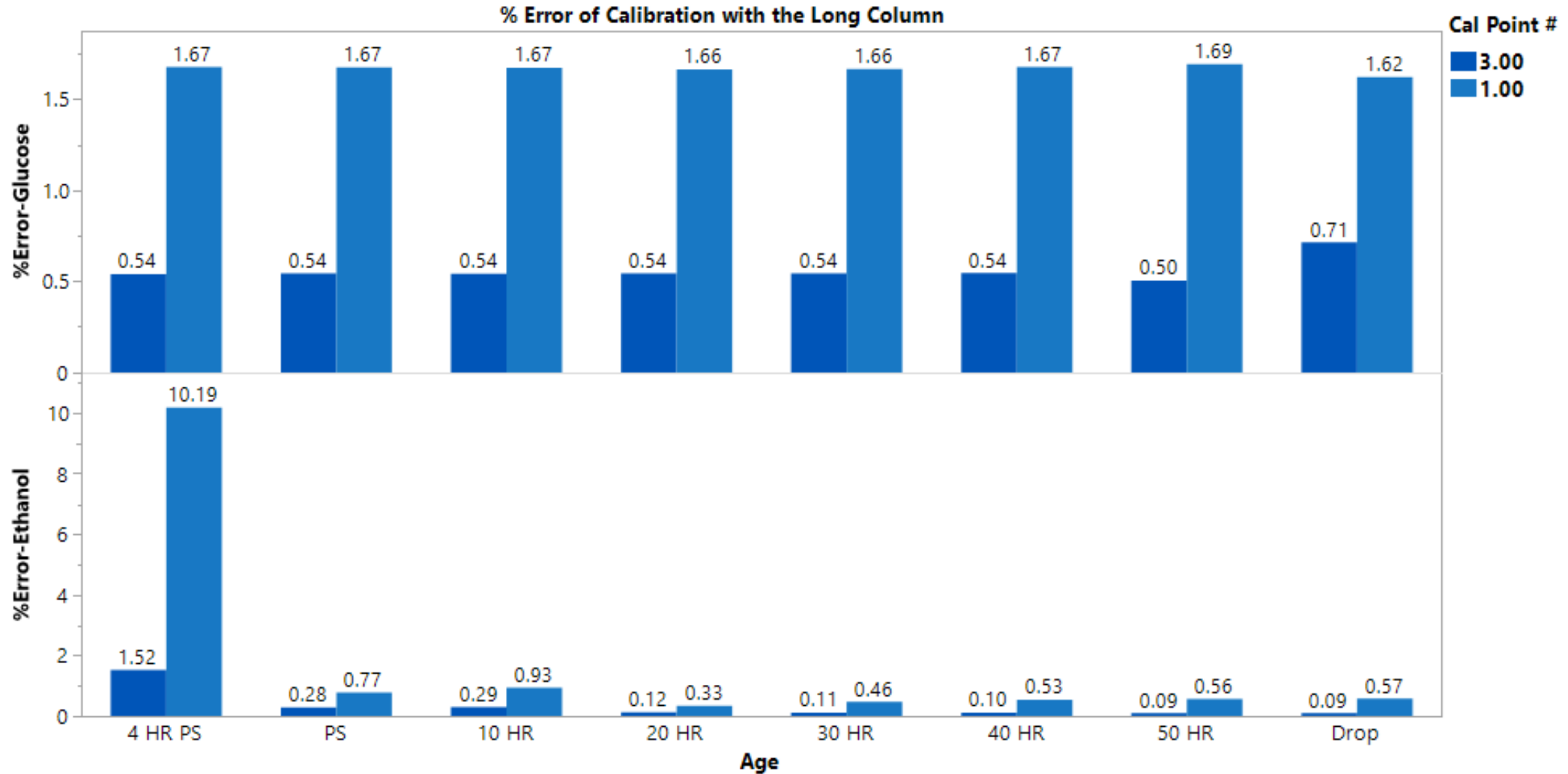


Ensures Quality Control: Monitoring % error ensures the HPLC system is performing reliably, crucial for maintaining accuracy in critical applications like the fermentation process.

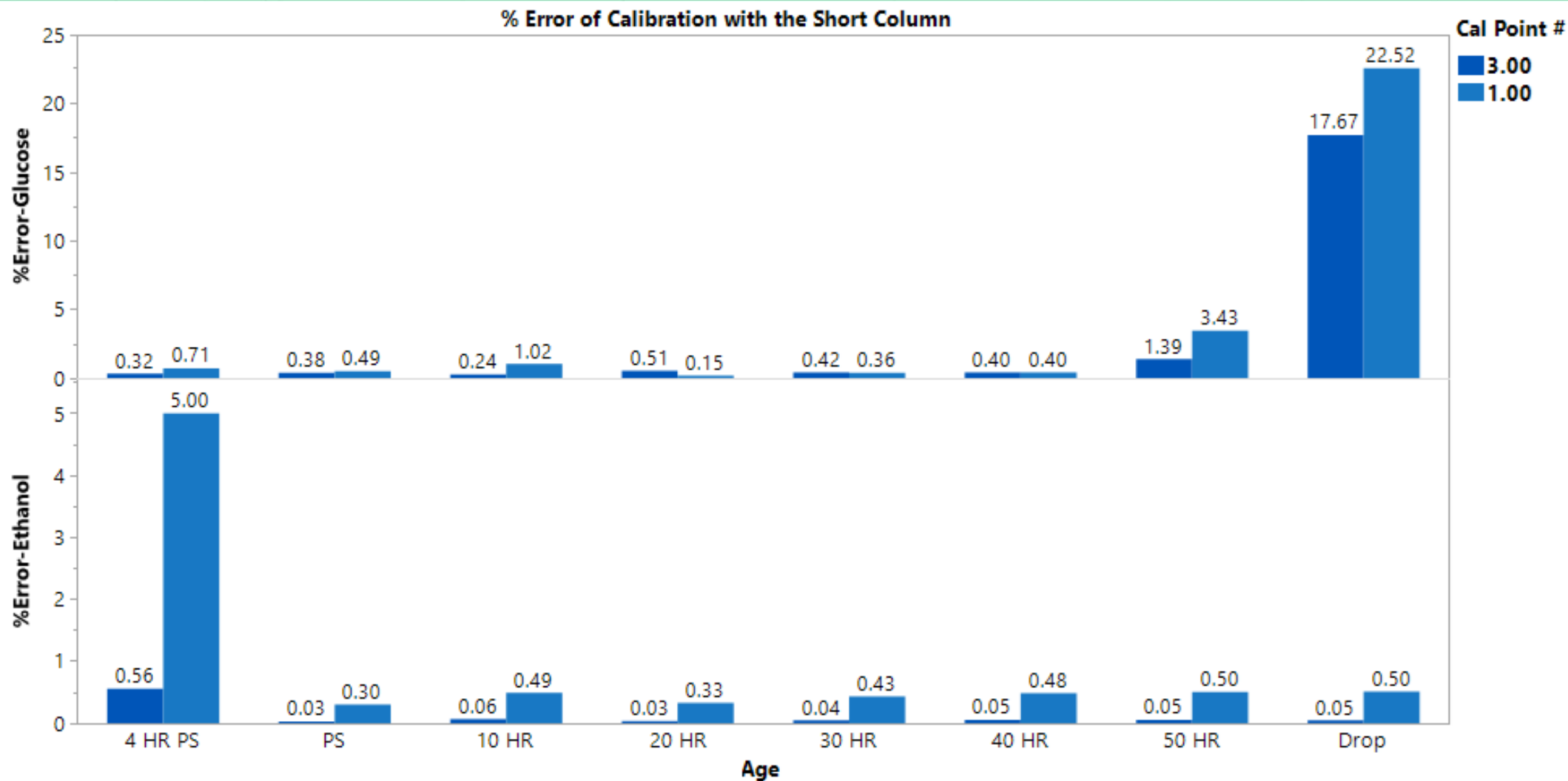
$$\% \text{ Error} = \frac{|V_{True} - V_{Observed}|}{V_{True}} \times 100$$



% Error Calibration Curve- Long Column



% Error Calibration Curve- Short Column

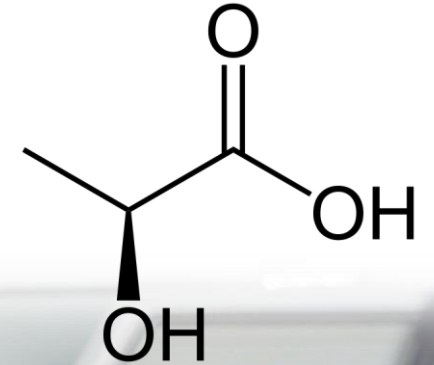


Case Study: Monitoring Lactic Acid Concentrations During an infection.

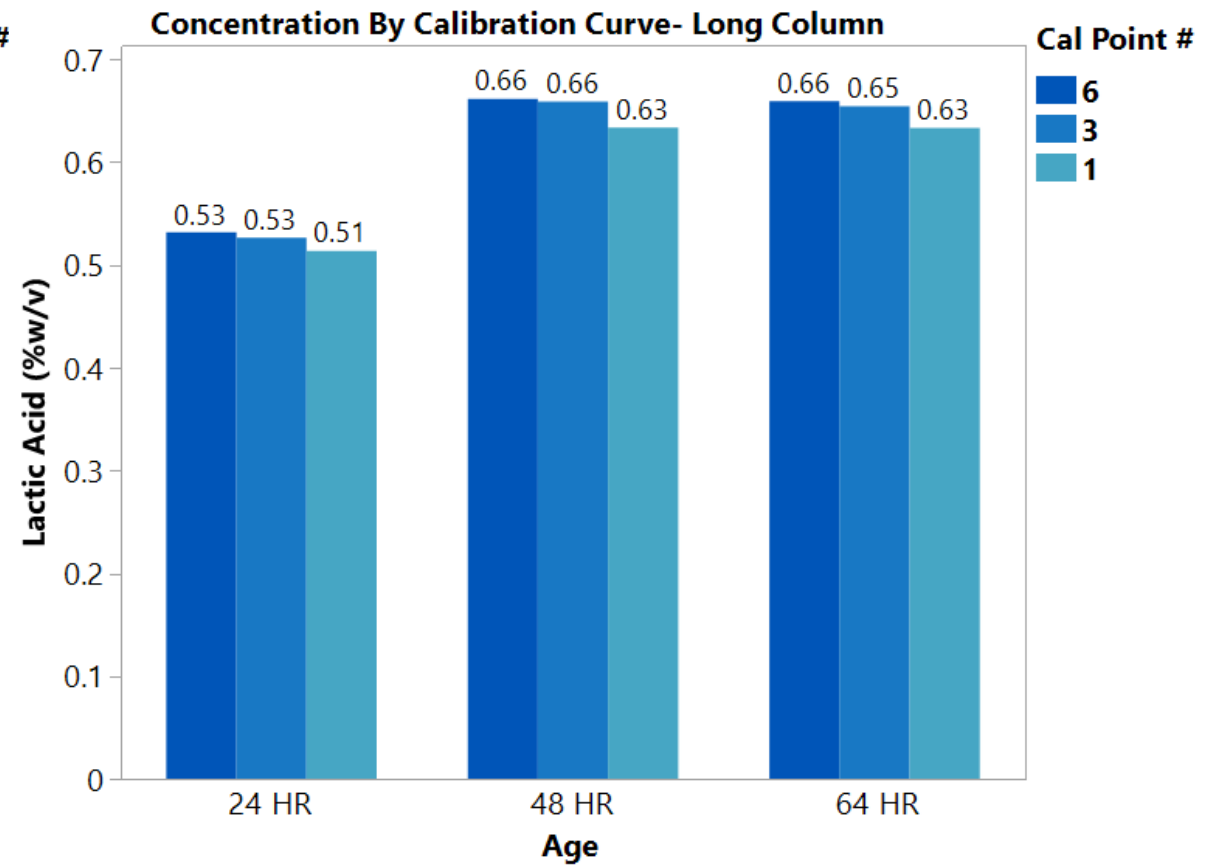
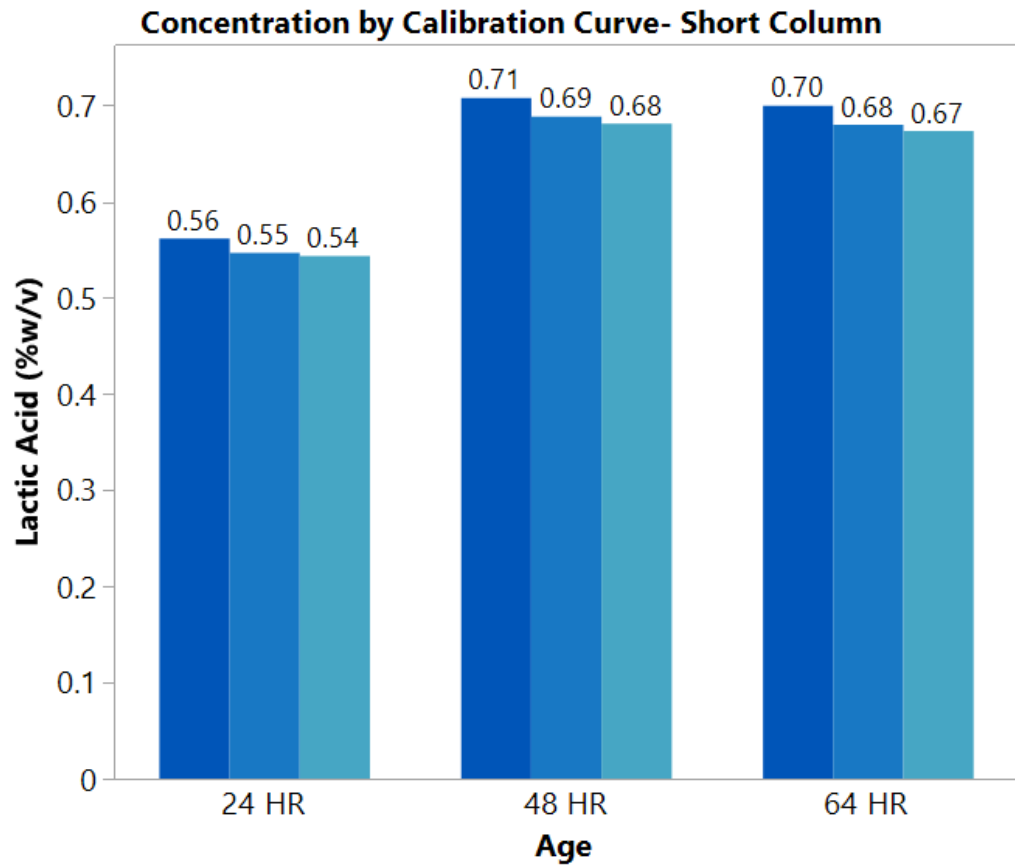
- Benchtop samples of infected mash were ran to simulate real-world scenarios.
- Lactic acid concentrations were measured using a Short Column (150 mm) and Long Column (300 mm) using 6-point, 3-point, and 1-point calibration curves.
- Comparative analysis of concentration values and % error was conducted across each calibration method.

Objective:

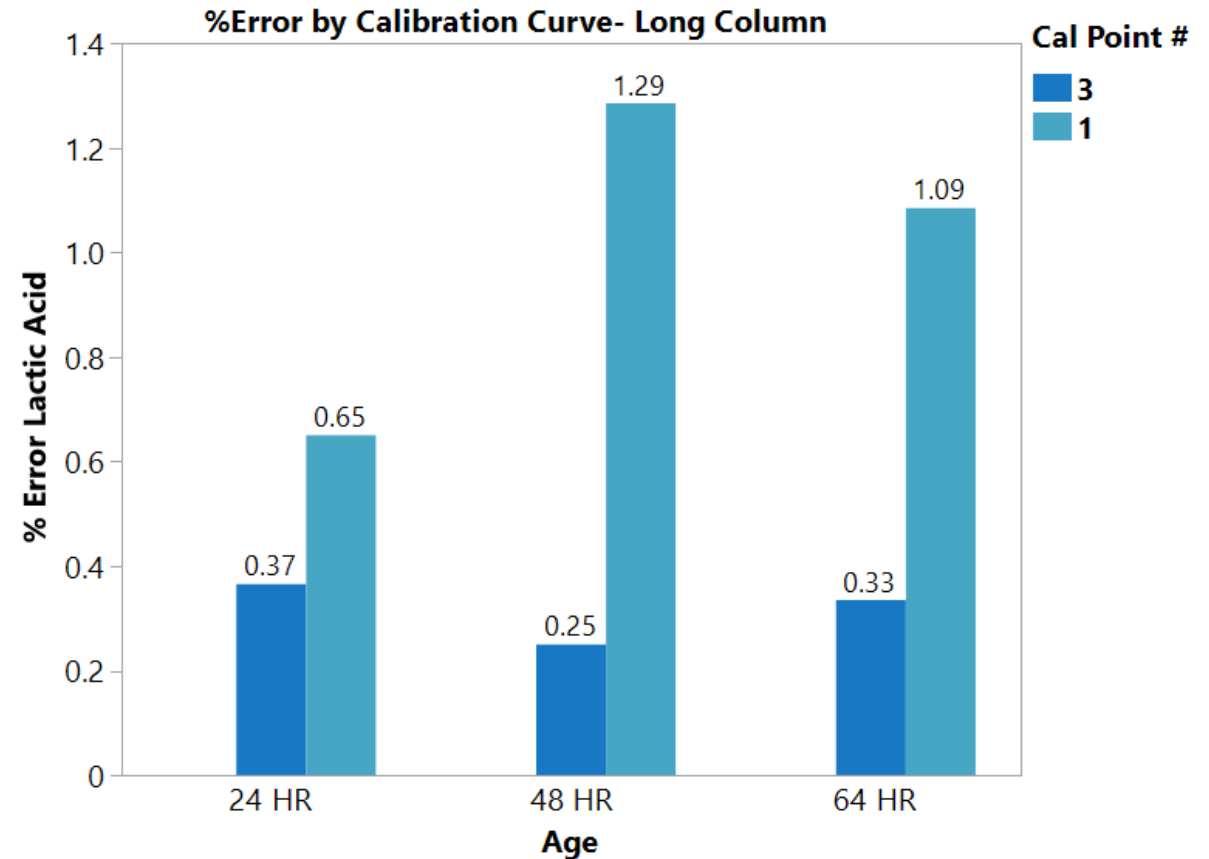
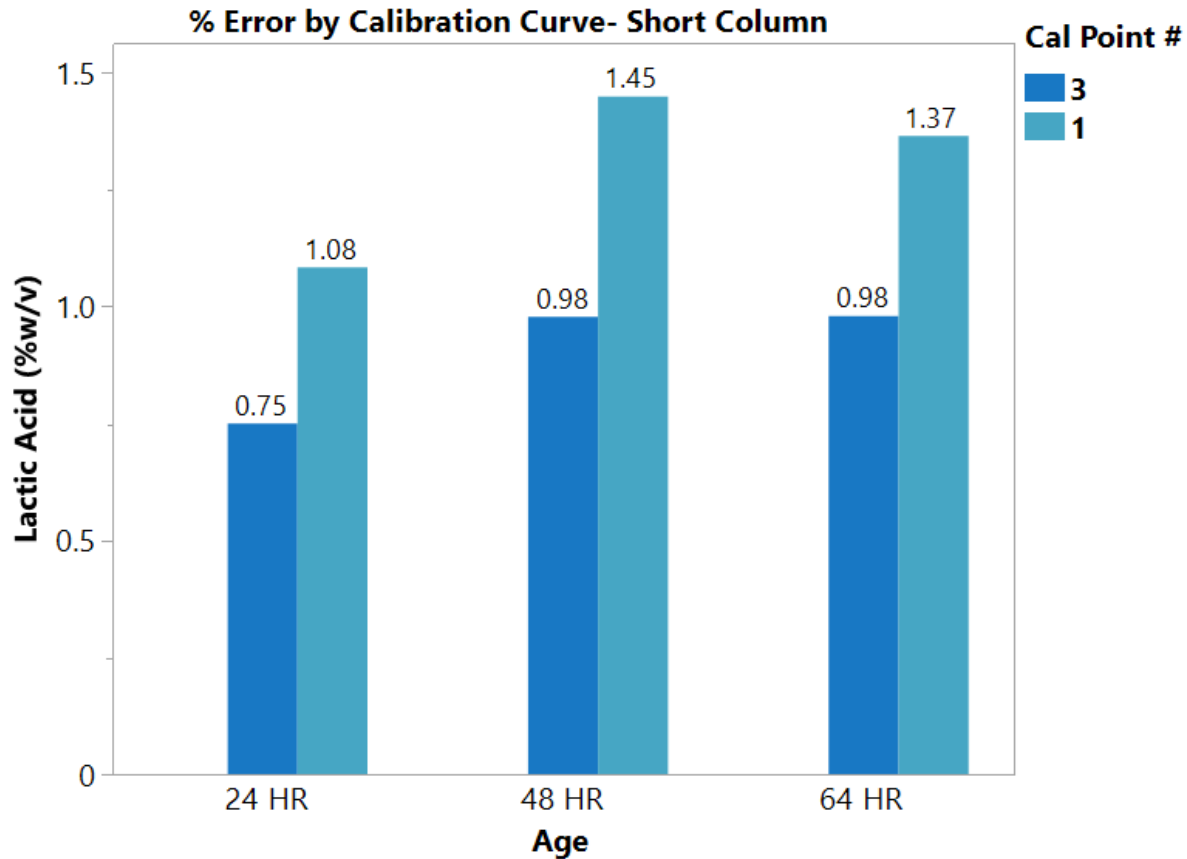
1. How does the use of different columns and calibration curves reflect lactic acid values during an infection, supporting more informed decision-making?
2. How does sugar conversion reflect similar trends with higher Lactic values?



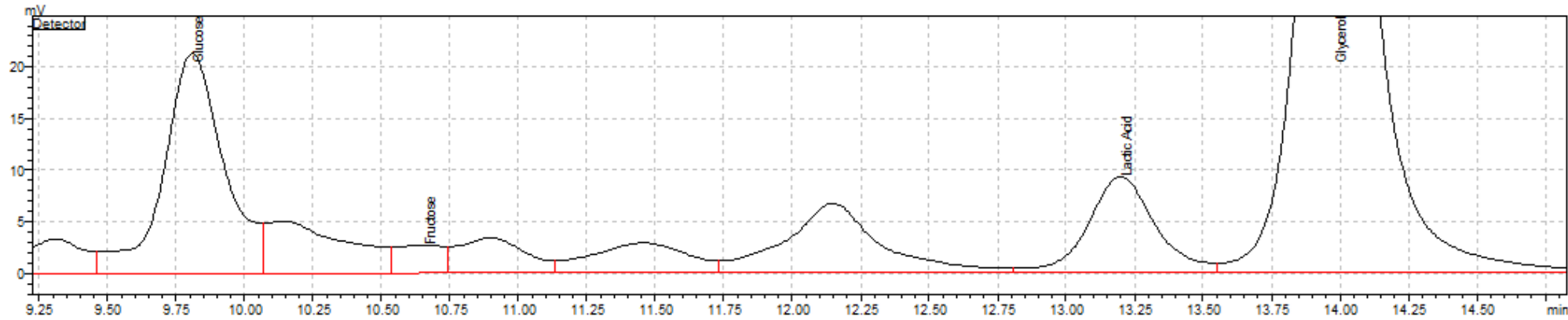
Lactic Acid Concentration Between Calibration



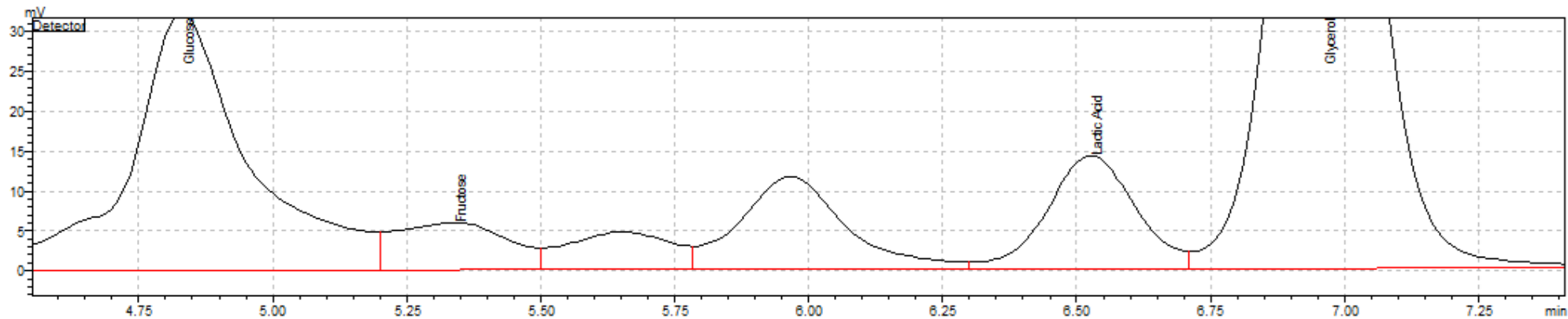
Lactic Acid % Error Between Calibration



Visual Comparisons- 64-Hour Sample

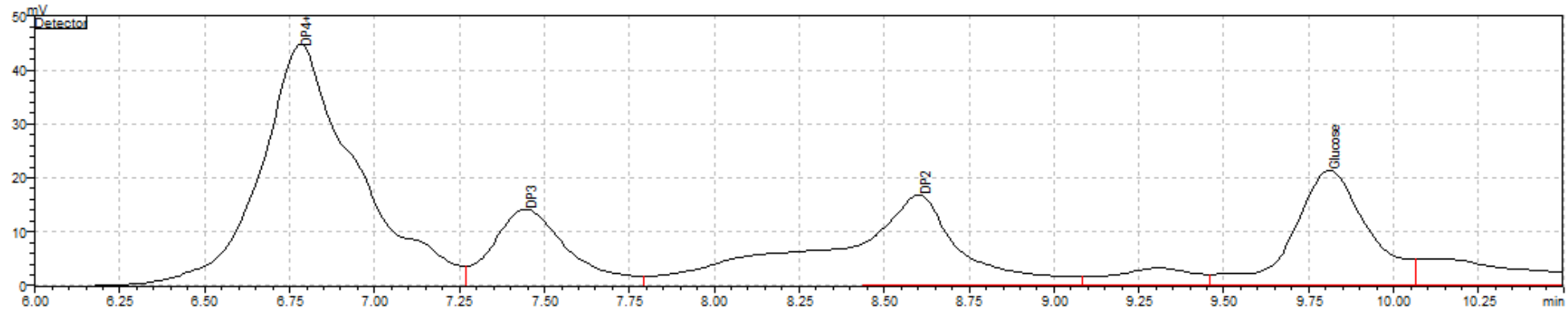


300mm Column

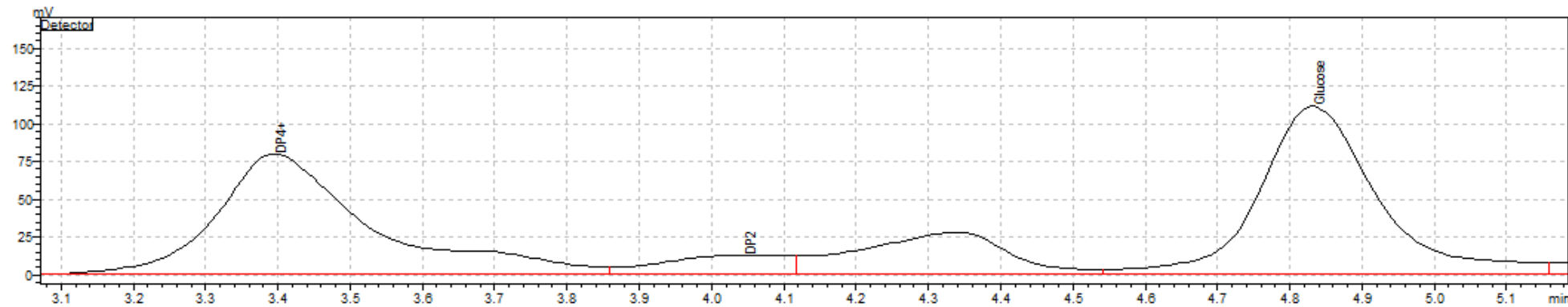


150 mm Column

Visual Comparisons- 64-Hour Sample



300mm Column



150 mm Column

Summary

- **Loss of Resolution:**
 - Reduced resolution leads to overlapping peaks:
 - Challenges in distinguishing between different compounds accurately, like lactic acid and glucose, especially during infection detection.
 - Can mask true concentrations, leading to inaccurate assessment
- **Implications of Resolution loss:**
 - Fermentation is Not Finishing:
 - Suggest incomplete fermentation due to high glucose concentrations.
 - Production Issues:
 - Potentially leads to unnecessary adjustments and inefficiencies.
- **Calibration points:**
 - Using more data points in calibration (e.g., multi-point curves) offers a better reflection of the relationship between concentration and response.
 - This approach increases accuracy and precision compared to single-point calibration, which can introduce inaccuracies, especially in complex analyses like fermentation monitoring



Possible Solutions

Resolution Considerations :

- **Recognizing Limitations:**

- Shorter columns may provide insufficient resolution for complex mixtures.
- Proposed Solutions: Utilizing longer columns can help improve resolution and enhance the separation of compounds.

- **Calibration Considerations:**

- Multi-Point Calibration:
 - Implementing 3- to 6-point calibration curves offers better representation across different analyte concentrations, improving precision and reducing error.
- Monitoring Instrument Parameters:
 - Regular checks of key instrument settings and knowing when to recalibrate ensure accurate and reliable results.



Thank you!

