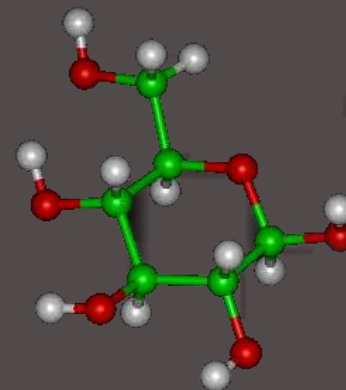


Improved Options for Sugar Analysis by HPLC

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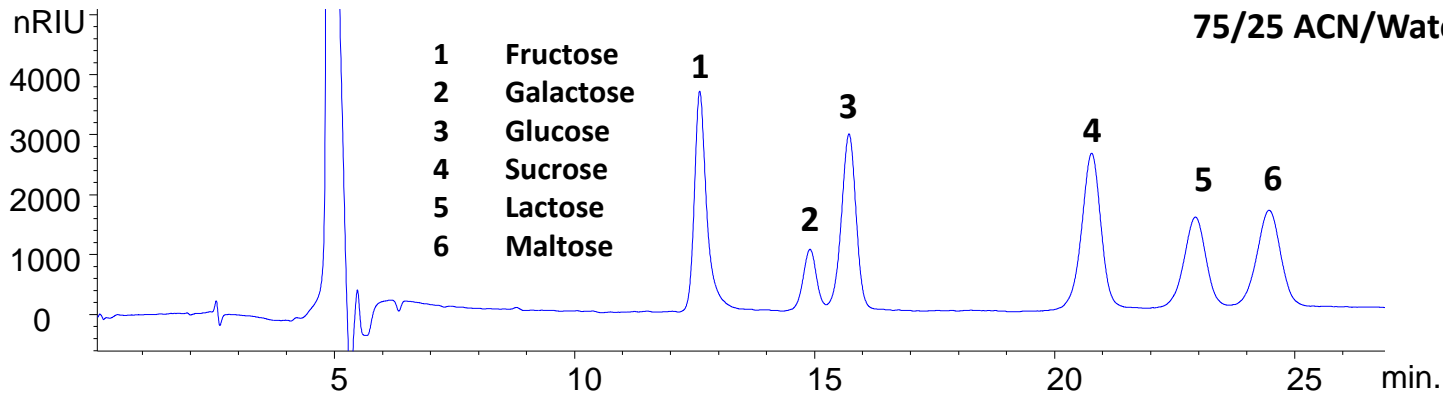


MARCH 1-5, 2020
CHICAGO, IL

Current Options for Separating Sugars by HPLC

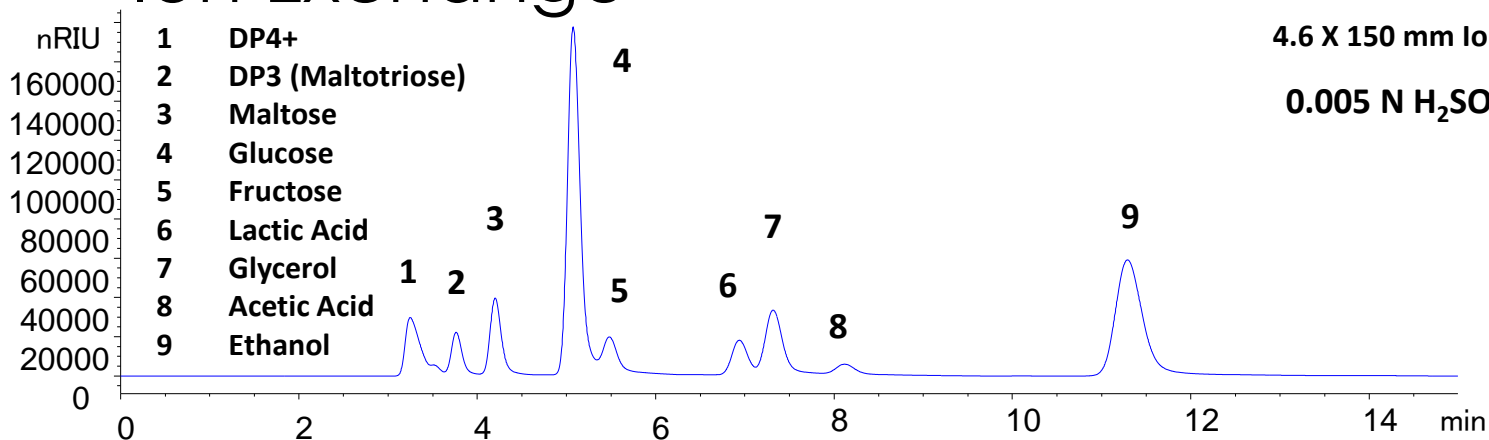
• HILIC/Amino Column

2X 4.6 X 150 mm APHera NH2
(2 columns in series)
75/25 ACN/Water



• Ion Exchange

4.6 X 150 mm Ion Exchange (H+)
0.005 N H₂SO₄

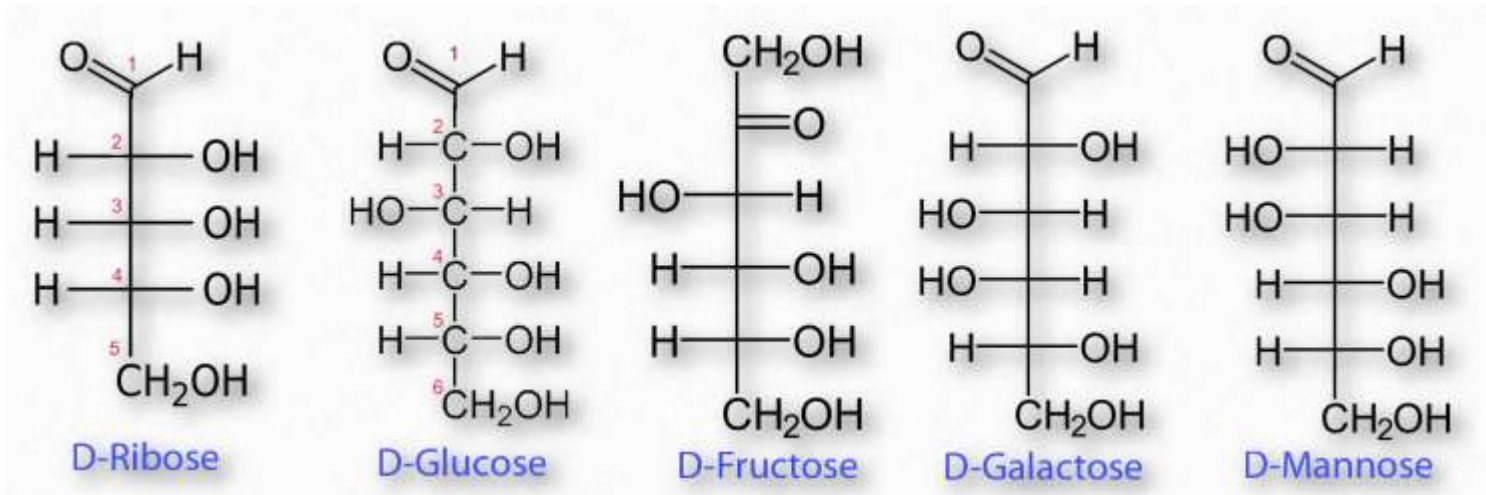
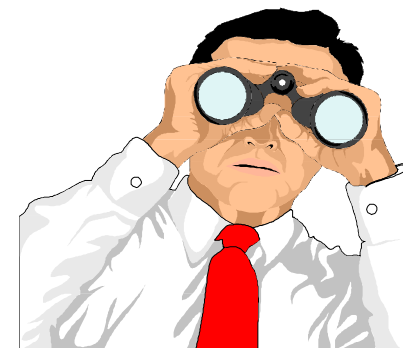


The Ideal Sugar Method

- Primary
 - Stable column/retention times
 - Short analysis time
 - High efficiency
 - Adequate reproducibility
- And It Would Be Nice If It Had
 - Gradient compatibility
 - Moderate/High Sensitivity
 - “Green” mobile phase



Looking For a Better Option



Organic Chemistry

“Like-Dissolves-Like”



Chromatography

“Like-Interacts With-Like”

Evaluation System

- Columns
 - Halo Pentahilic (OH_x)
 - 4.6X 50 mm, 2.7 μm
 - 3.0 X100 mm, 2.7 μm
- Mobile Phase
 - Water/Formic Acid/Ammonium Formate
 - Organic solvents – ACN, Methanol, IPA, THF, Acetone
- HPLC
 - Agilent 1290
 - Binary pump
 - High performance autosampler
 - Column heater
 - ELSD

Why this column?

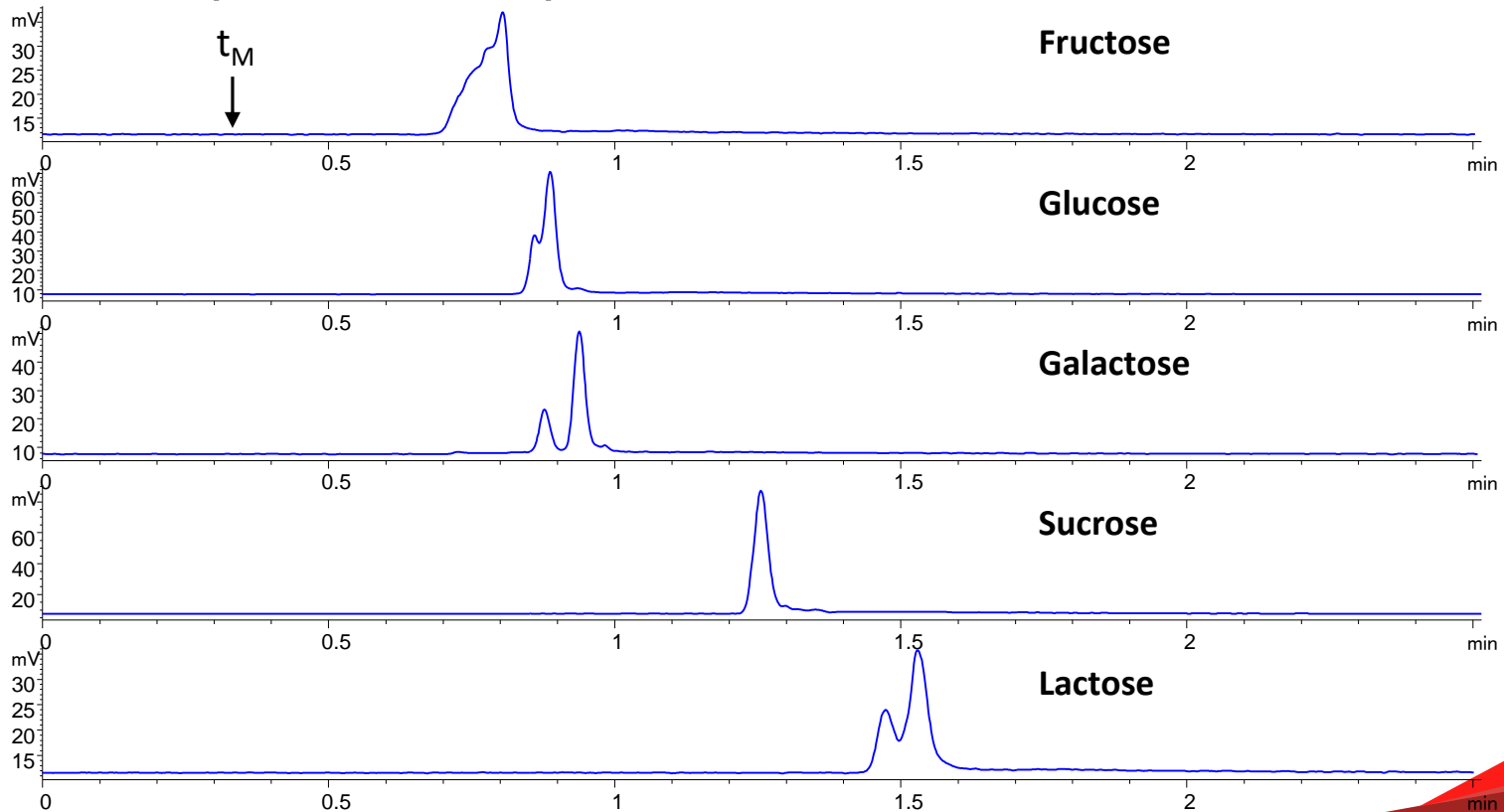
The stationary phase is essentially a five carbon sugar-like molecule. A “fructan” column.



Initial Results

- Split peaks for all but sucrose
- Poor peak shape for fructose

In 80% ACN



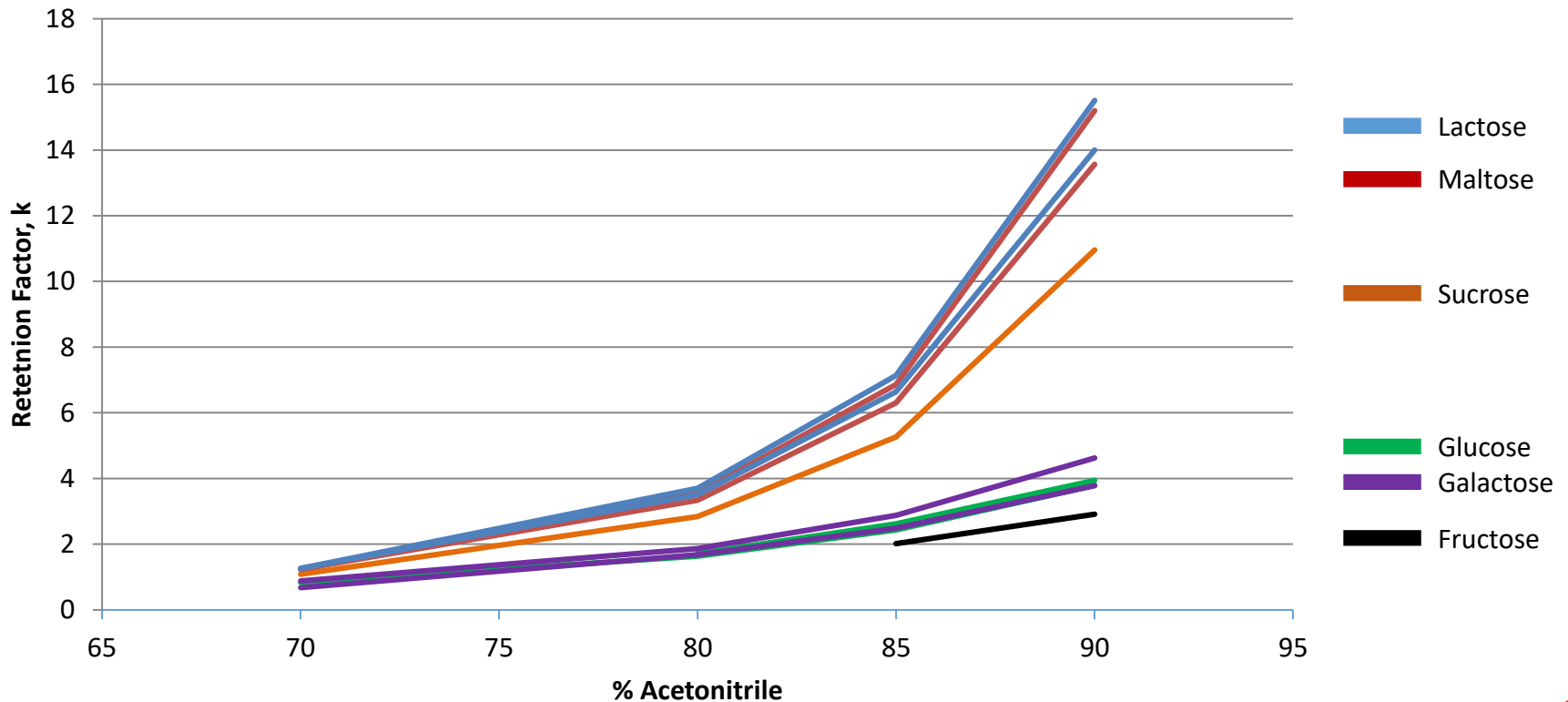
What about other modifiers?

- Would other HILIC “weak” solvents work better?
 - Evaluated
 - Acetone
 - THF
 - IPA
 - Methanol
 - All produced *no significant retention*.
 - Ternary mixtures with these solvents at $\leq 10\%$ levels produced *less retention with no change in selectivity or peak splitting*.
- *What about pH modifiers?*
 - *Neither formic acid or ammonium formate produced significant changes.*
- **So, acetonitrile/water it is!**

Retention Pattern

Column: 3.0X 100 mm, 2.7 μ m
Mobile Phase: H₂O/ACN
 Flow: 0.75 mL/min
 Injection: 1 μ L
 Column Temperature: 35 C
 Detector: ELSD [40 °C, 45 psi]
 Sample: 1.0 mg/mL

- Retention behavior follows a typical HILIC trend



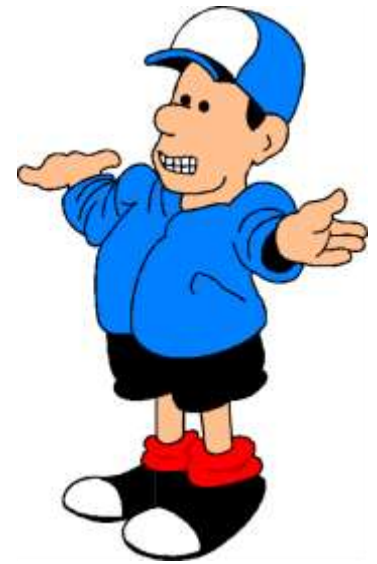
Behavior Summary

- No “greener” mobile phase options (at this time)
- Small sugars exhibit the usual HILIC retention trend with acetonitrile
- No benefit from pH adjustments
- Split peaks for most small sugars
 - Separation increases with retention



- Simple water/acetonitrile conditions can produce short analysis times with good resolution for selected sugars

So Where Do We Go From Here?



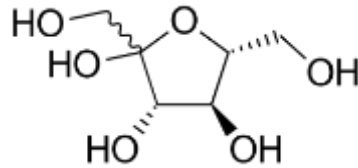
- Focus on operating conditions that minimize retention ($k < 5$)
 - Minimize peak splitting
 - Adjust for minimum acceptable resolution
 - Isocratic for simple mixtures, or gradients if a wide range of sugars is present
- Secondary benefit is short analysis times

Fruit Drinks

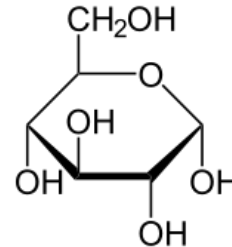


- Primary sugars are

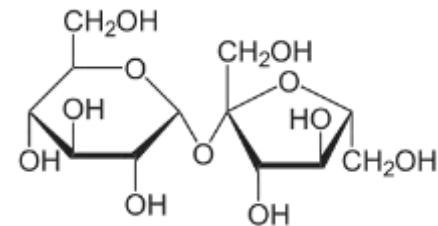
- Fructose



- Glucose

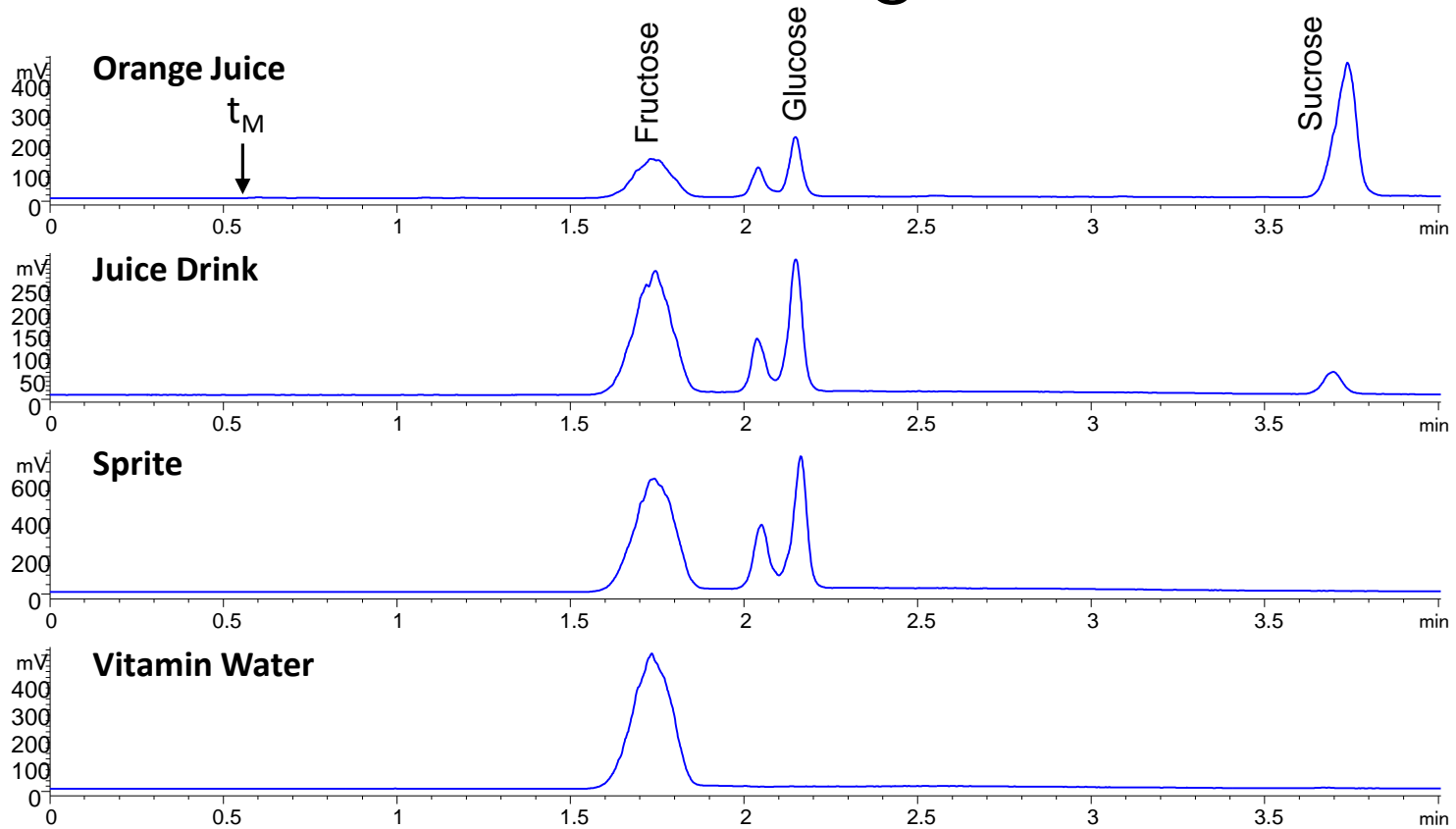


- Sucrose (Glucose-Fructose)



Results: Fruit Drinks

- Four minute method with good resolution.

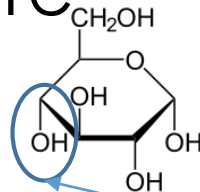


Dairy Products

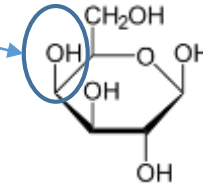


• Primary sugars are

- Glucose

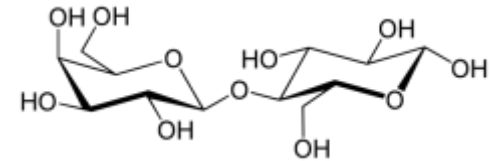


- Galactose



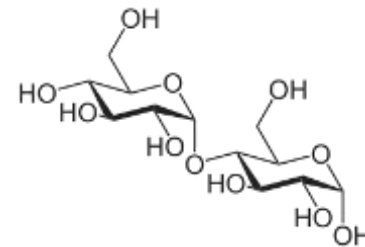
- Lactose (Glucose-Galactose)

- Dairy-Based Only



- Maltose (Glucose-Glucose)

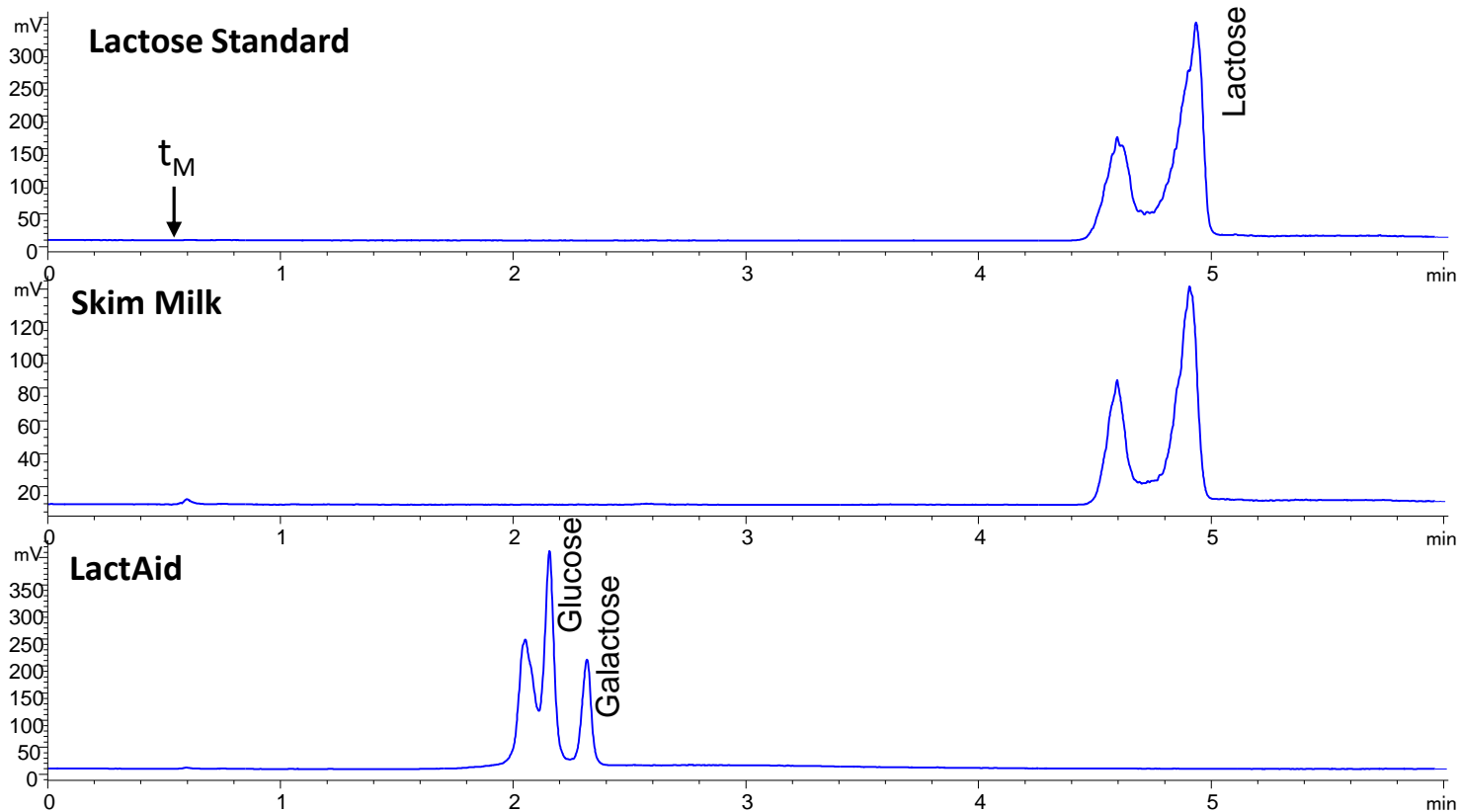
- Plant-Based Only



Results: Dairy/ Plant Drinks

Column: 3.0X 100 mm, 2.7 μ m
Mobile Phase: H₂O/ACN (15/85)
 Flow: 0.75 mL/min
 Injection: 2 μ L
 Column Temperature: 35 C
 Detector: ELSD [40 °C, 45 psi]
 Sample: Diluted 1:10 with water/acn

- Six minute method with no interferences.



Speeding Things Up

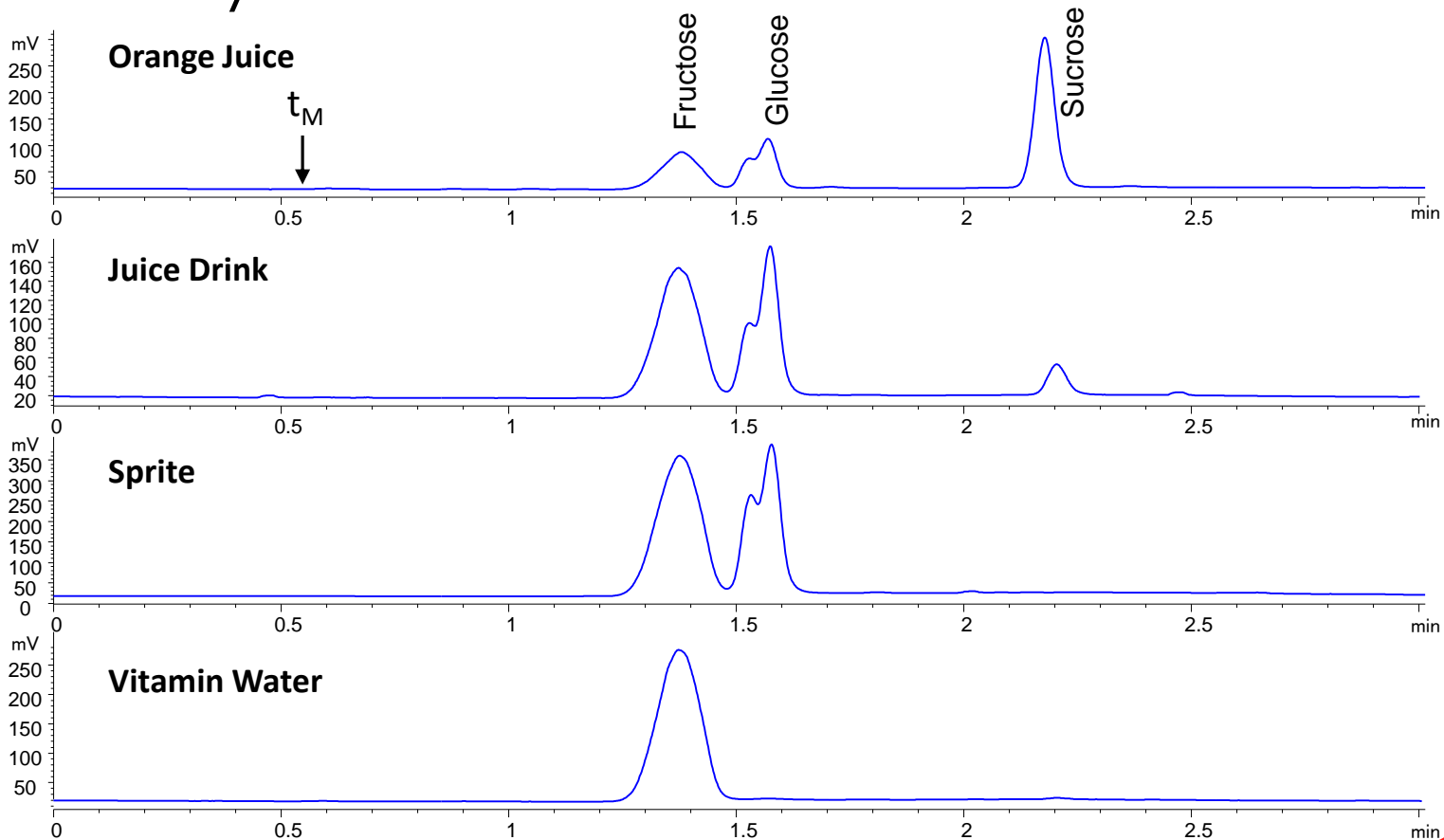


- Reduce retention to minimize separation between peaks
 - Reduce k with more water
 - 20 % for fruit samples
 - 25 % for dairy/plant samples
- Adjust data acquisition settings
 - Data Rate: 10 Hz → 5 Hz
 - Response Time: 0.5 sec → 2 sec
 - Minimize detector noise and help merge split peaks

Fruit Drinks (Fast Method)

Column: 3.0X 100 mm, 2.7 μ m
 Mobile Phase: H₂O/ACN (20/80)
 Flow: 0.75 mL/min
 Injection: 2 μ L
 Column Temperature: 35 C
 Detector: ELSD [40 °C, 45 psi]
 Sample: Diluted 1:10 with water/acn

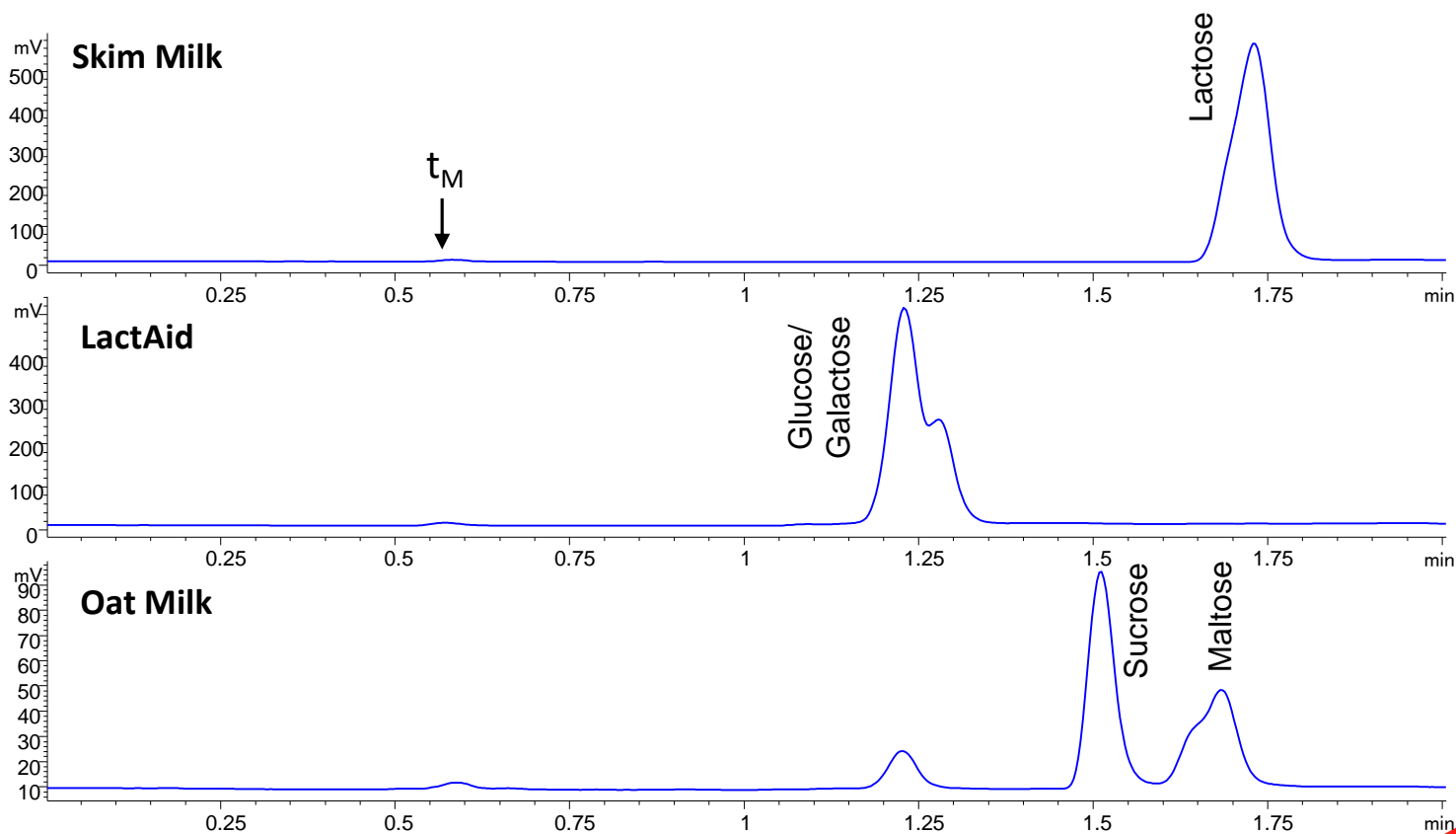
- Analysis time less than 2.5 minutes!



Dairy/Plant Drinks (Fast Method)

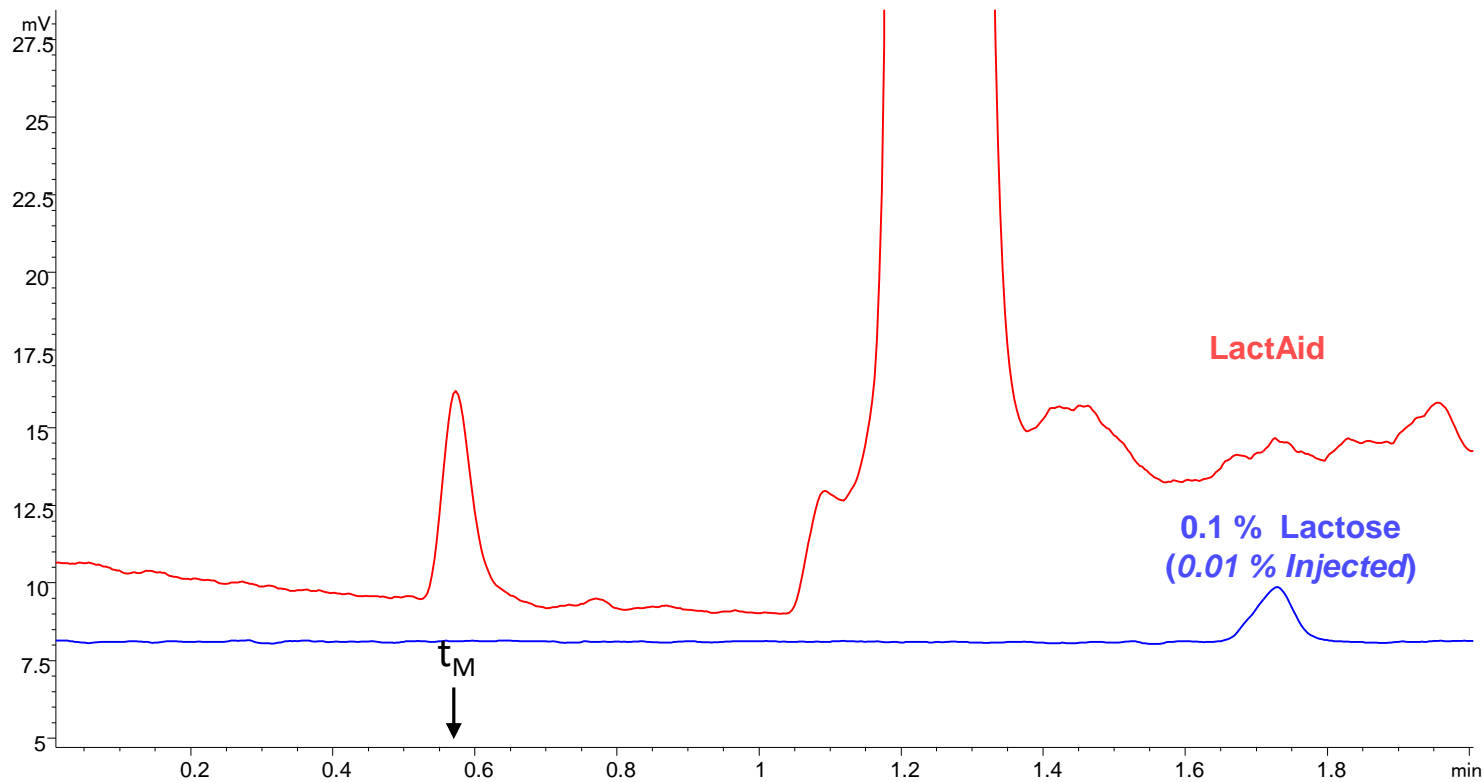
Column: 3.0X 100 mm, 2.7 μ m
 Mobile Phase: H₂O/ACN (25/75)
 Flow: 0.75 mL/min
 Injection: 1 μ L
 Column Temperature: 35 C
 Detector: ELSD [40 °C, 45 psi]
 Sample: Diluted 1:10 with water/acn

- Analysis time less than 2 minutes!



Sensitivity Example

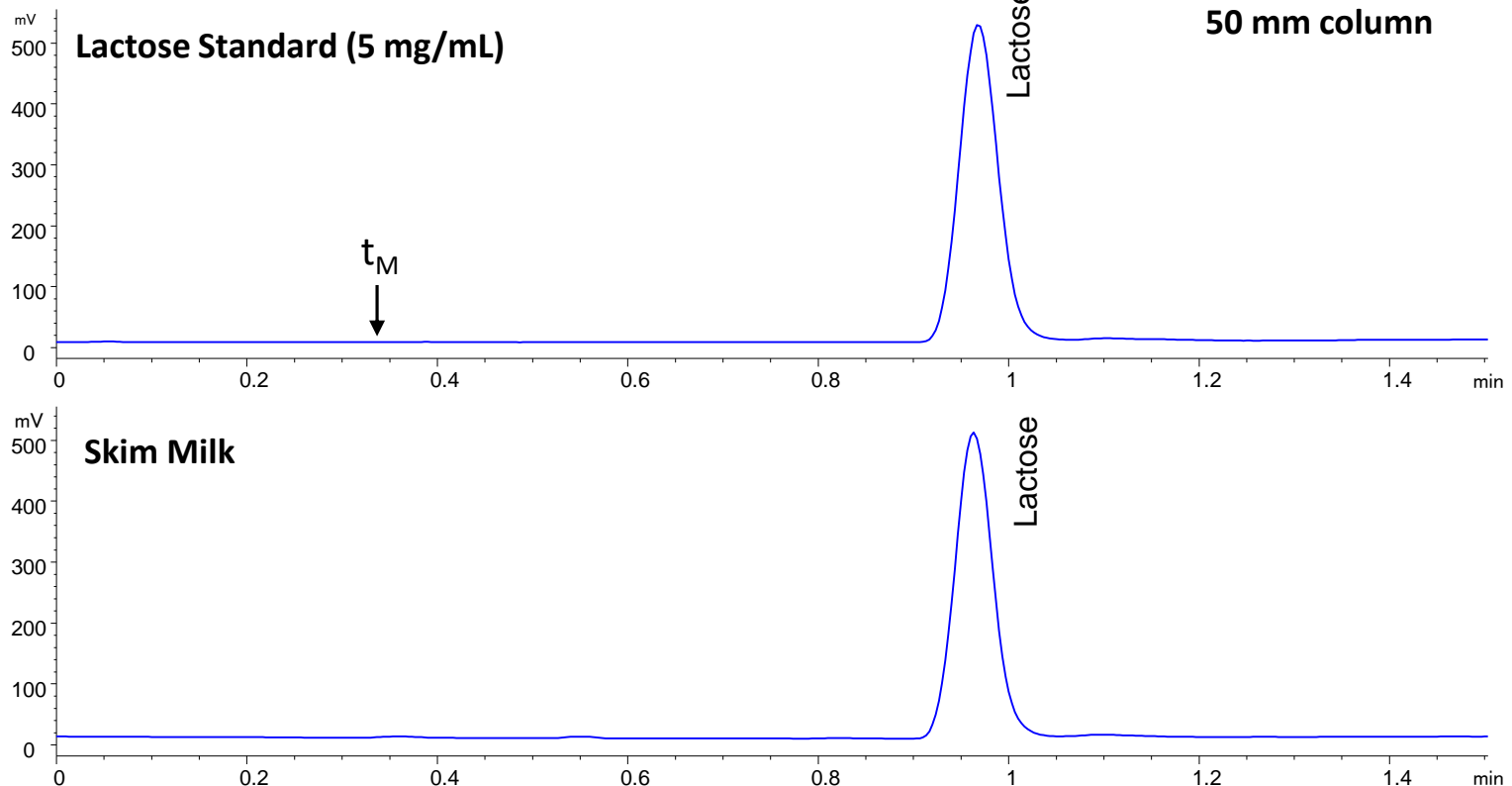
- To be “Lactose Free,” the product must contain $<0.1\%$ lactose.



Lactose in Milk

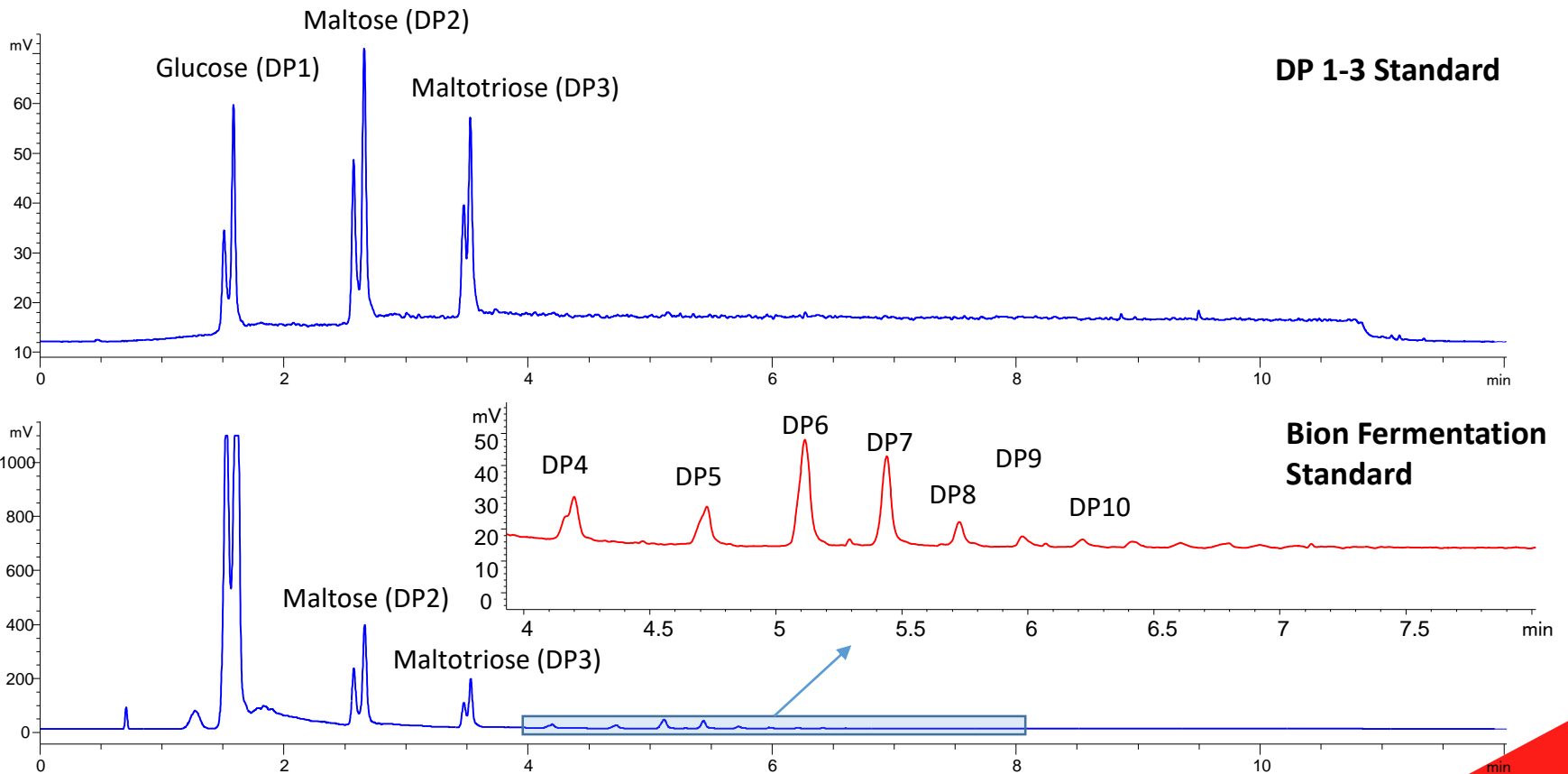
Column: 4.6X 50 mm, 2.7 μ m
Mobile Phase: H₂O/ACN (25/75)
Flow: 1.5 mL/min
Injection: 1 μ L
Column Temperature: 35 C
Detector: ELSD [40 °C, 45 psi]
Sample: Diluted 1:10 with water/acn

- If the injected sample is simple (no interferences), even faster separations are possible with a shorter column.



DP Sugars

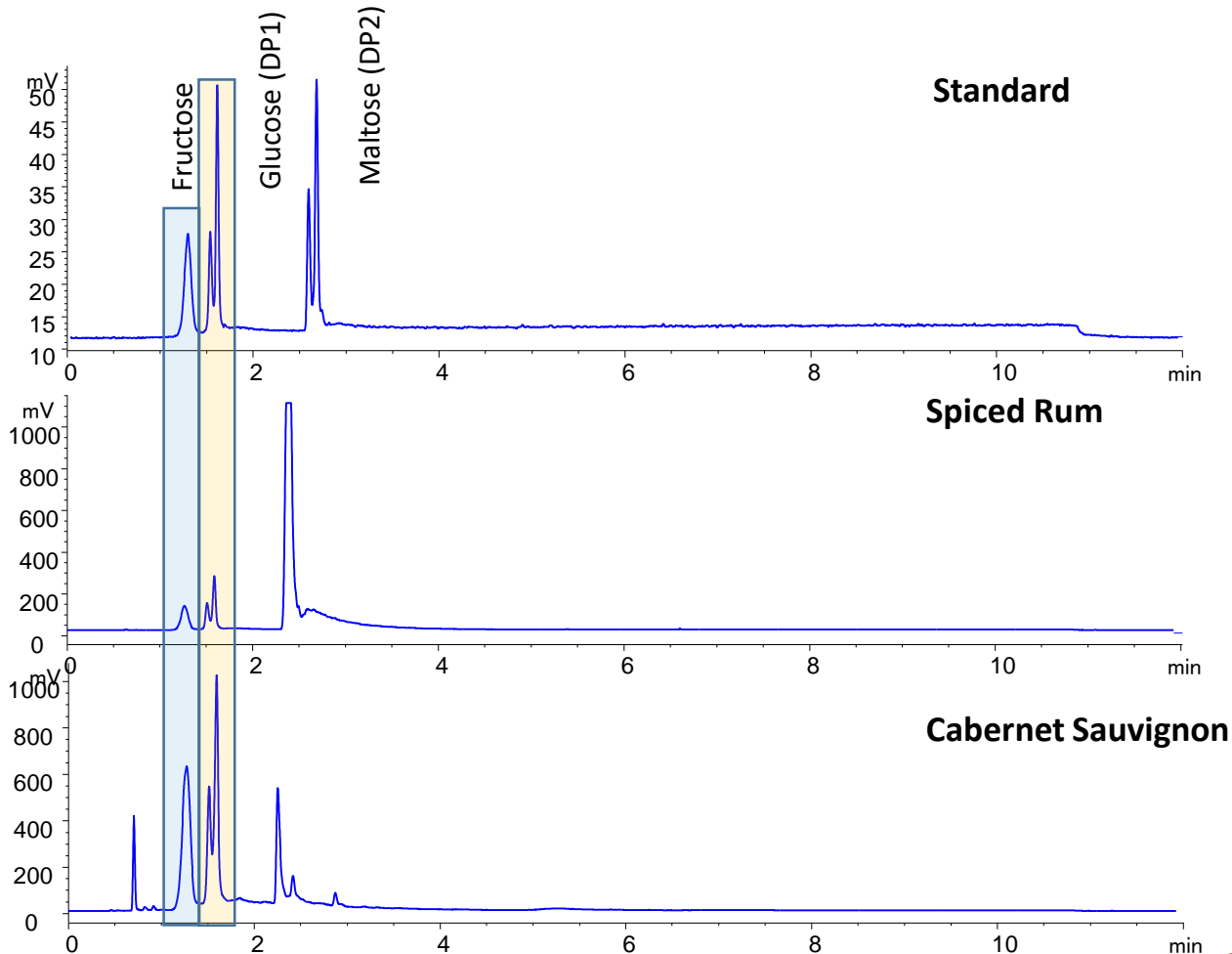
- Gradient elution on a fructan column allows determination of higher level DP sugars



Sugar Profiles in Other Beverages

Column: 4.6X 50 mm, 2.7 μ m
Mobile Phase: H₂O/ACN (90-40% ACN/10 min.)
Flow: 1.5 mL/min
Injection: 1 μ L
Column Temperature: 35 C
Detector: ELSD [40 °C, 45 psi]
Sample: Filtered

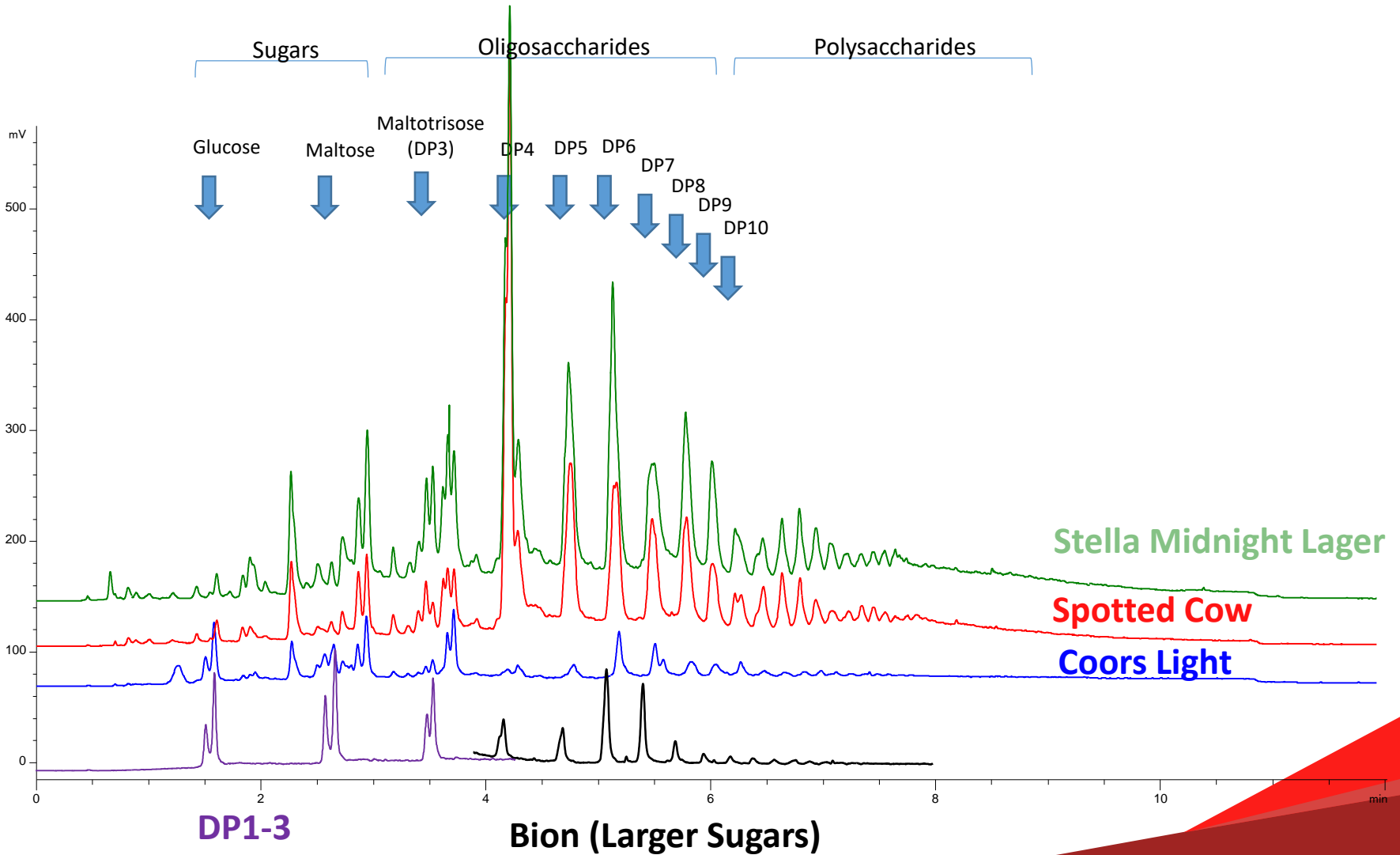
- Direct injection produces simple sugar profile





Beer Is More Interesting

Column: 4.6X 50 mm, 2.7 μ m
Mobile Phase: H₂O/ACN (90-40% ACN/10 min.)
Flow: 1.5 mL/min
Injection: 1 μ L
Column Temperature: 35 C
Detector: ELSD [40 °C, 45 psi]
Sample: Filtered



Summary

- Limitations
 - Glucose and Galactose overlap.
 - Lactose and Maltose overlap.
 - Split peaks are not aesthetically desirable, but they can be integrated as one peak.
- Advantages
 - Fast isocratic methods
 - Simple mobile phase
 - Good sensitivity
 - Gradients offer some advantages
 - Reduce peak splitting
 - Extend range to larger sugars



Acknowledgements

- Advanced Materials Technologies
 - Richard A. Henry, Ph. D.
 - Thomas J. Waeghe, Ph.D.

Questions?

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