

Comparison of Positive and Negative Ionization-Based Electrospray Methods for Analyzing Cellodextrins in Biomass Samples

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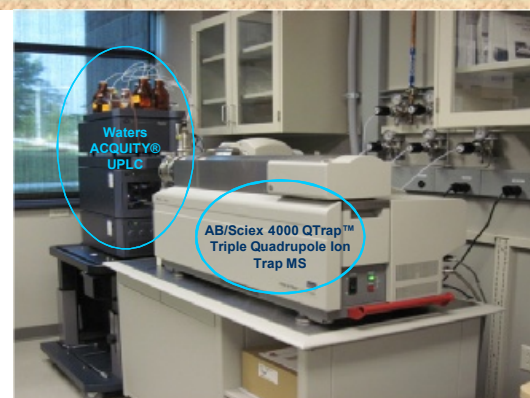
OVERVIEW

- A new method for identifying and measuring cellodextrins (carbohydrates) employing UPLC/negative-ionization electrospray MS detection was compared to a similar method employing positive ionization.
- Maltodextrin standards were used to extend the comparison to $5 \leq n \leq 7$ sugar oligomers.
- The new method was capable of detecting the higher oligomers, but at the expense of sensitivity.
- Both methods, when challenged with common unknowns, provided similar, but not identical, quantitative and qualitative data.

INTRODUCTION

- Identifying and quantifying carbohydrates in a sample of biomass is critical for understanding the conversion of raw plant-based material, such as switchgrass or poplar, to ethanol.
- Our "existing" analytical method for selected cellodextrins separates compounds using a BEH HILIC UPLC column in six minutes and uses positive electrospray ionization.¹
- The method is satisfactory for smaller ($4 \geq n \geq 2$) oligomers, but is unsatisfactory for oligomers where $n > 5$.
- A newer "candidate" method separates compounds using a BEH Amide UPLC column and employs negative electrospray ionization.²
- A wider range of related isomeric maltodextrin oligomers ($7 > n > 1$) is resolved, and should be suitable for the current purpose.
- This presentation tests both methods head-to-head using maltodextrin and cellodextrin standards and biomass samples.

APPARATUS



POSITIVE IONIZATION ELECTROSPRAY METHOD

The dextrins were separated using a 2.1 mm i.d. x 100 mm UPLC® BEH HILIC column employing a 0.5 µL injection volume. A gradient separation employing acetonitrile (10 µM sodium acetate, Solvent A) and water (10 µM sodium acetate, Solvent B) was employed, as shown below. The sample-to-sample analysis time was 6 min.

Time, min	% A (v/v)	% B (v/v)	Flow rate, mL/min
Initial	80	20	0.5
1.0	80	20	0.5
2.0	70	30	0.5
2.1	5	95	0.5
3.0	5	95	0.5
3.1	80	20	0.5

The dextrins were detected as their corresponding sodiated analogs, m/z 365.0, 527.1, 689.1, 851.1, 1013.5, and 1175.5 Da, for the maltose oligomers. The AB/Sciex 4000 Qtrap employed electrospray ionization in the positive ionization mode. All ions were monitored in the single-ion monitoring (SIM) mode. The instrument was optimized for detection of the seven target m/z values.

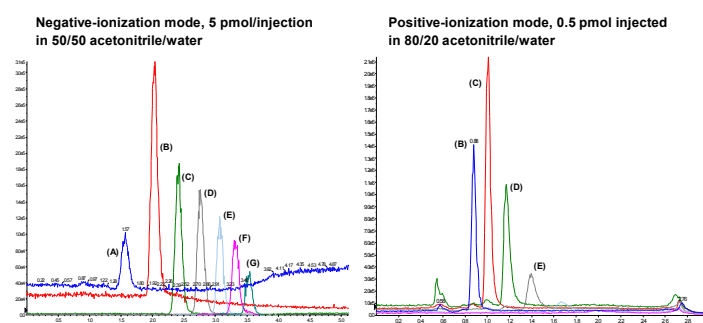
NEGATIVE IONIZATION ELECTROSPRAY METHOD

The dextrins were separated using a 2.1 mm i.d. x 50 mm UPLC® BEH Amide column employing a 1 µL injection volume. A gradient separation employing 80/20/0.1 acetonitrile/water/NH₄OH (Solvent A) and 30/70/0.1 acetonitrile/water/NH₄OH (Solvent B) was employed, as shown below. The sample-to-sample analysis time was 10 min.

Time, min	%A (v/v)	%B (v/v)	Flow rate, mL/min
Initial	80	20	0.17
5.0	10	90	0.17
6.1	90	20	0.17
10.0	80	20	0.17

The dextrins were detected as their corresponding (M-H)⁻¹ negative ions, m/z 178.9, 341.0, 503.0, 665.1, 827.1, 989.20, and 1151.2 Da, for the maltose oligomers. The AB/Sciex 4000 QTrap employed electrospray ionization in the negative ionization mode. All ions were monitored in the SIM mode. The instrument was optimized for detection of the seven target m/z values.

COMPARISON OF RESULTS FROM STANDARDS



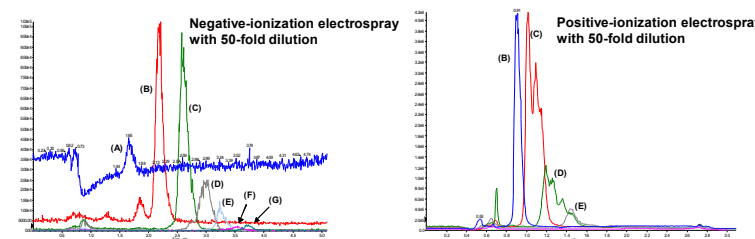
The positive-ionization mode is ten-fold more sensitive than the negative-ionization mode for maltodextrins, but the higher oligomers are detected more easily in the latter. The negative-ionization mode also permits the detection of fructose isomers.

Legend: (A) fructose; (B) maltose; (C) maltotriose; (D) maltotetraose; (E) maltopentaose; (F) maltohexose; (G) maltoheptaose.

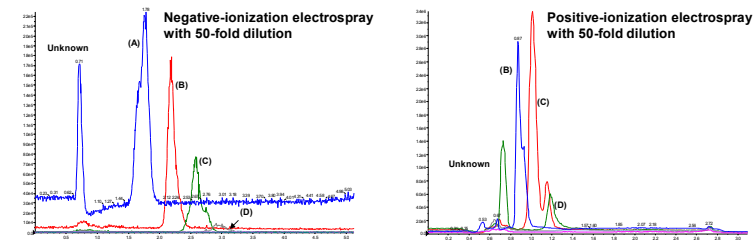
Compound	Detection Limit, fmol	
	Positive Ionization	Negative Ionization
Cellulobiose	30	95
Maltose	75	160
Cellotriose	17	93
Maltotriose	40	110
Cellotetraose	28	120
Maltotetraose	53	120
Cellopentaose	86	170
Maltopentaose	87	70
Maltohexaose	n/a	240
Maltoheptaose	n/a	180

Detection limits for the cellodextrins and maltodextrins were determined independently for the positive ionization mode (0.025-0.50 pmol injected) and the negative-ionization mode (0.05-2.5 pmol injected). The detection limits were estimated from the linear calibration curves ($3 s_{xy}/\text{slope}$, where s_{xy} , the standard error of the y value estimates, is assumed to approximate the standard deviation of the blank, s_b). Calibration curves were constructed using six standards spanning the stated range, with five-fold replication for each value.

COMPARISON OF RESULTS FROM BIOMASS SAMPLES



Side-by-side comparison from Sample A12 (Avicel®, pure cellulose) at the same dilution shows more oligomers using the negative ionization method, but more resolved species using the positive ionization method.



Side-by-side comparison from Sample C12 (degraded cellobiose) at the same dilution shows more oligomers using the negative ionization method, but more resolved species using the positive ionization method.

Sample Name and Dilution	Analyte Concentration, µM			
	Cellulobiose		Cellotriose	
	Mean	Std Dev	Mean	Std Dev
A01 1/50	36.3	1.4	24.0	1.9
A01 1/50	44.3	<10	27.4	<10
A02 1/50	83.8	3.1	33.0	2.9
A02 1/50	129.7	24.6	35.9	<10
A11 1/50	90.8	3.3	71.2	4.7
A11 1/50	64.8	<10	156.0	24.4
A12 1/50	83.9	10.4	68.3	7.7
A12 1/50	74.8	11.6	177.5	24.8

Both methods exhibit comparable accuracy and precision based on identical unknown samples. Black numbers represent positive-ionization MS; red numbers represent negative-ionization MS. All values based on five-fold replication

CONCLUSIONS

Which method is better? And, why?

Criterion	Positive Ionization	Negative Ionization
Additives		✓
Solvent consumption		✓
Linear range		✓
Oligomer range		✓
Detection limits	✓	
Speed	✓	
Start-up time	✓	
Level of detail	✓	
Signal-to-noise ratio	✓	
Robustness	✓	?

- The positive ionization method is preferred when the analytes are $2 \leq n < 5$ and high sensitivity is desired over a 25-fold linear dynamic range.
- The negative ionization method is preferred when the primary objective is to examine samples for oligomers $1 \leq n$ over a 50-fold linear dynamic range.
- The limited number of unknown samples available (12) make any evaluation of the robustness of the negative ionization method unreliable. By contrast, we have used the positive ionization method successfully for two years covering approximately 200 samples.
- The absence of proper biomass reference materials makes it difficult to determine which method produces accurate quantitative and qualitative information for a given unknown sample.
- Stable isotope internal standards, which would improve the quantitation, are either very expensive (e.g., sucrose-¹³C₁₂) or nonexistent.
- The reliability of the quantitation could be improved by using the method of standard addition or other related technique.

ACKNOWLEDGMENTS

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¹ B. A. Tomkins, G. J. Van Berkel, T. J. Tschaplinski, and N. L. Engle, "Evaluation of the Cellodextrin Profiles of the Enzymatic Digests of Switchgrass", Poster TP108, presented at the 57th ASMS Conference on Mass Spectrometry and Allied Topics, May 31-June 4, 2009.

² K. J. Fountain, C. Hudalla, D. McCabe, and D. Morrison, "UPLC-MS Analysis of Carbohydrates", Waters Corporation, October 2009.