UPLC® and CE Methods for Orthogonal Analysis of N-Glycans

A summary of Ultra-Performance Liquid Chromatography (UPLC) and Capillary Electrophoresis (CE) methods used in the ProZyme poster, Orthogonal Methods for Glycoanalysis: CE and UPLC (CE Pharm 2013)[1]

UPLC Methods

Analytical System

LC System: Waters ACQUITY UPLC® (H-class) with FLR detector, Empower™ software

Columns

- Waters ACQUITY UPLC BEH Glycan 1.7 µm (Glycan Separation Technology, GST column)

Method	Column Size	Waters Part Number
5-Minute Gradient	2.1 x 50 mm	186004740
10-Minute Gradient	2.1 x 100 mm	186004741
60-Minute Gradient A[2,3]	2.1 x 150 mm	186004742
60-Minute Gradient B	2.1 x 150 mm	186004742

- No guard column was used
- Column Temperature: 60°C

Sample Preparation Recommendations

- Prepare samples following protocols set forth in corresponding manual for GlykoPrep® Rapid N-Glycan Preparation with 2-AB (ProZyme product code GP96NG-AB).
- Inject 1 µl of aqueous sample per UPLC run.
- Remaining samples may be stored frozen in the dark. Use sealing film if storing in 96-well plates.

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Solvents

- Solvent A: Acetonitrile
- Solvent B: 100 m*M* Ammonium Formate pH 4.4

Excitation and Emission

Excitation: 360 nm
Emission: 428 nm

5-Minute Gradient

Method Details

Time (min)	Flow Rate (ml/min)	% A	% B
0	1.5	73	27
4	1.5	55	40
4.05	1.5	30	60
4.25	1.5	30	60
4.3	1	73	27
4.7	1.5	73	27
5	1.5	73	27

10-Minute Gradient

Method Details

Time (min)	Flow Rate (ml/min)	% A	% B
0	1	75	25
8	1	60	40
8.1	0.5	40	60
8.5	0.5	40	60
8.6	1	40	60
8.8	1	75	25
10.0	1	75	25

60-Minute Gradient A[2,3]

Method Details

Time (min)	Flow Rate (ml/min)	% A	% B
0	0.5	78	22
38.5	0.5	55.9	44.1
39.5	0.25	20	80
44.5	0.25	20	80
46.5	0.5	78	22
60	0.5	78	22

60-Minute Gradient B

Method Details

Time (min)	Flow Rate (ml/min)	% A	% B
0	0.75	75	25
54	0.75	62.5	37.5
55	0.25	40	60
56	0.25	40	60
57	0.5	75	25
58	0.75	75	25
60	0.75	75	25

CE Methods

Analytical System

CE System: Beckman Coulter® PA800 *plus* with LIF detector, 32-Karat™ software

Capillaries

- Beckman Coulter N-CHO and eCAP (developmental)

Method	Capillary	Beckman Part Number
10-Minute Separation[4]	eCAP	NA
15-Minute Separation[6]	N-CHO	477601
20-Minute Separation[2]	N-CHO	477601
35-Minute Separation[5]	N-CHO	477601

Sample Preparation Recommendations

- Prepare samples following protocols set forth in corresponding manual for GP96NG-APTS
- Load each sample at 2 psi for 10 seconds
- Remaining samples may be stored frozen in the dark.

Separation Buffers

Method-specific; see individual methods

Excitation and Emission

Excitation: 360 nm
Emission: 428 nm

10-Minute Separation[4]

Method Details

Length	60 cm (effective: 50 cm)
I.D.	30 µm
Separation Buffer	25 m*M* boric acid, 8 m*M* ammonium acetate; pH 9.0
Temperature	35°C
Voltage	30 kV for 10 minutes
Polarity	Reversed

15-MinuteSeparation[6]

Method Details

Length	60 cm (effective: 50 cm)
I.D.	50 µm
Separation Buffer	40 m*M* ϵ-aminocaproic acid, 40 m*M* acetic acid, 0.2% hydroxymethylcellulose
Temperature	20°C
Voltage	30 kV for 15 minutes
Polarity	Reversed

20-Minute Separation[2]

Method Details

Length	60 cm (effective: 50 cm)
I.D.	50 µm
Separation Buffer	N-linked Carbohydrate Separation Gel Buffer (Part No. 477623)
Temperature	20°C
Voltage	30 kV for 20 minutes
Polarity	Reversed

35-Minute Separation[5]

Method Details

Length	60 cm (effective: 50 cm)
I.D.	50 µm
Separation Buffer	1:1 Mixture of N-linked Carbohydrate Separation Gel Buffer (Part No. 477623) and eCAP ds DNA 1000 Gel Buffer (Part No. 477628)
Temperature	20°C
Voltage	20 kV for 35 minutes
Polarity	Reversed

References

1. Szabo *et al.* Orthogonal Methods for Glycoanalysis: CE and UPLC. Poster presented at: CE in Biotechnology & Pharmaceutical Industries: 15th Symposium on the Practical Applications for the Analysis of Proteins, Nucleotides and Small Molecules (CE Pharm 2013), October 6-10, 2013, Arlington, VA, USA.

2. N-Glycan Mapping Study Using Orthogonal Methods: CE and UPLC. Workshop at CE in Biotechnology & Pharmaceutical Industries: 15th Symposium on the Practical Applications for the Analysis of Proteins, Nucleotides and Small Molecules (CE Pharm 2013), October 6-10, 2013, Arlington, VA, USA.

3. Ahn, J. *et al.* UPLC-FLR Method Development of 2-AB-Labeled Glycan Separation in Hydrophillic Interaction Chromatography (HILIC). Application Note. Waters Corporation, Milford, MA, USA.

4. Szabo, Z. *et al.* Rapid High-Resolution Characterization of Functionally Important Monoclonal Antibody N-Glycans by Capillary Electrophoresis. Analytical Chemistry, 2011, 83, (13), 5329-5336.

5. Rampal, S. *et al.* Separation of Fucosylated, non-Fucosylated, and Complex Carbohydrates Associated with Monoclonal Antibodies using Capillary Electrophoresis. Chromatography Today 2011, 3, 38-40.

6. Hamm, M. *et al.* Characterization of N-Linked Glycosylation in a Monoclonal Antibody Produced in NS0 Cells Using Capillary Electrophoresis with Laser-Induced Fluorescence Detection. Pharmaceuticals, 2013, 6, 393-406.