Utilizing novel phenylpyridine tags for *N*-linked glycans profiling by capillary electrophoresis with LED-induced fluorescence and/or mass spectrometry detection

Denisa Smolkova[1,2], Michal Gregus[3], Richard Cmelik[1], Hana Pizova[3], Ross David Jansen-van Vuuren[4], Pavel Bobal[3], Jana Lavicka[1]

[1] Institute of Analytical Chemistry of the Czech Academy of Sciences, Veveri 97, 602 00 Brno, Czech Republic
[2] Masaryk University, Faculty of Science, Department of Chemistry, Kamenice 5, 625 00 Brno, Czech Republic
[3] Masaryk University, Faculty of Pharmacy, Department of Chemical Drugs, Palackeho trida 1, 612 00 Brno, Czech Republic
[4] University of Ljubljana, Faculty of Chemistry and Chemical Technology, Vecna pot 113, 1000 Ljubljana, Slovenia
lavicka@iach.cz

Introduction

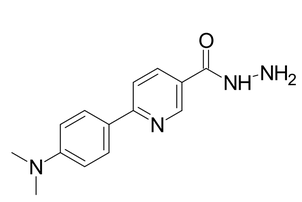
Glycosylation analysis is challenging due to the structural complexity and varied conjugation patterns of glycans, along with technical limitations like detection sensitivity. Therefore, a derivatization step is always required before analysis, especially by electrophoretic separation methods. Most derivatization approaches have utilized the negatively charged fluorophore 8-aminopyrene-1,3,6-trisulfonic acid trisodium salt, followed by capillary electrophoresis with laser-induced fluorescence (CE/LIF) and/or mass spectrometry (CE-MS) detection [1]. However, attaching a cationic tag with a high number of positive charges could enhance migration speed in CE, simplify the selection of separation buffers for electrophoretic sample concentration, and enable more sensitive detection by MS in positive ion mode.

Objectives

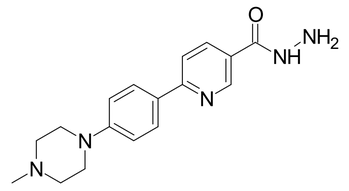
- **Introducing novel labeling reagents based on phenylpyridine with a hydrazide functional group, designed for glycan profiling using CE/LIF and/or CE-MS.**

Oligosaccharide/glycan labeling

Newly designed and synthesized labels

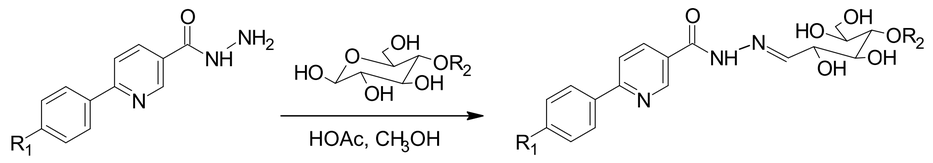


6-[4-(dimethylamino)phenyl]pyridine-3-carbohydrazide (DFP) [2]



6-[4-(4-methylpiperazin-1-yl)phenyl]pyridine-3-carbohydrazide (PFP)

Hydrazone formation chemistry

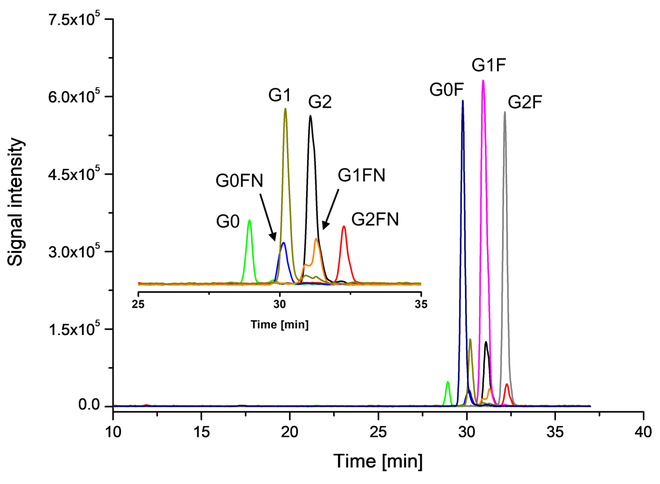


Labeling yield: ~ 90 % [3]

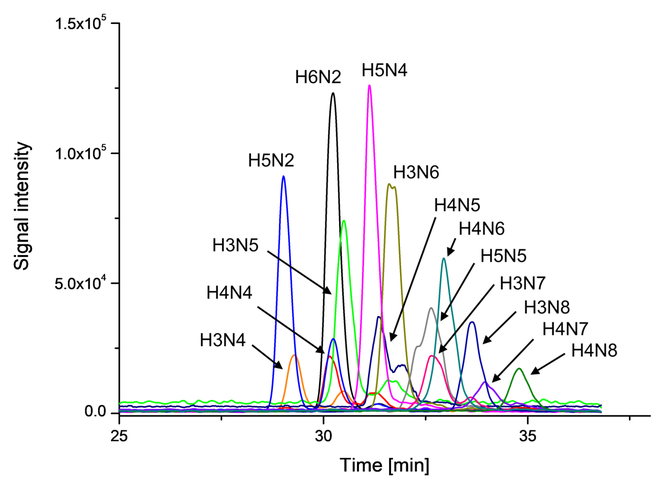
Labeling conditions
oligosaccharide:label ratio, 1:10 molar ratio
90 % (v/v) methanol + 10 % (v/v) acetic acid
50 °C, 5h

CE-MS analysis of DFP-labeled *N*-linked glycans

Human immunoglobulin G glycans



Chicken albumin glycans



CE
Agilent 7100 CE instrument
50 µm ID capillary, 70 cm, LPAA coating
BGE: 1M acetic acid, 30 kV

MS
Bruker maXis impact (ESI-TOF/MS)
nanoCEasy interface [4]
Sheath liquid: 1 % (v/v) formic acid + 50 % (v/v) propan-2-ol, 1.7 kV

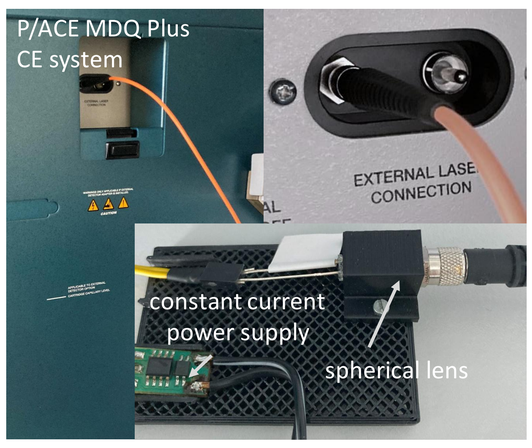
RSDs of migration times, peak heights, and peak areas of DFP-labeled maltooligosaccharide standards were determined to be 1.91–2.11 %, 5.66–8.27 %, and 4.18–7.04 %, respectively.
LODs were determined to be 10–28 nM (S/N = 3, n = 5).

CE/LED-IF analysis of PFP-labeled maltooligosaccharides

CE
Sciex P/ACE MDQ Plus system
100 µm ID capillary, 40 cm, LPAA coating
BGE: 250 mmol/L formic acid + 50 % (v/v) methanol, 30 kV

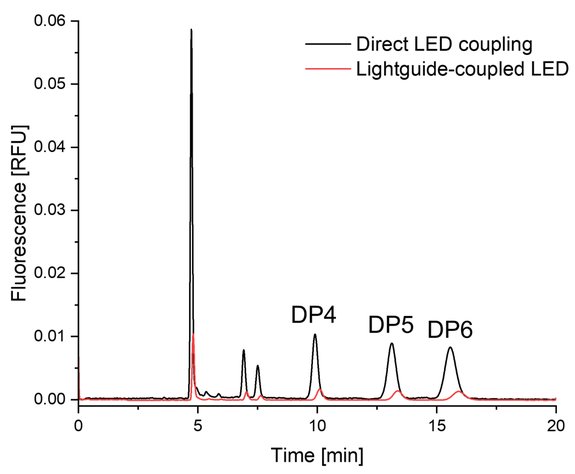
LED-IF
Excitation source: 340 nm LED (0.9 mW)
Emission filter: 370 nm longpass filter

Lightguide-coupled LED - 3D printed adapter

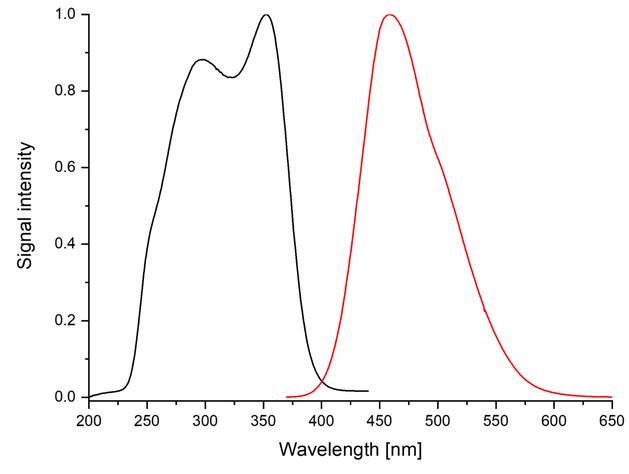


Direct LED coupling - machined aluminum adapter

Maltooligosaccharides (DP4-DP6)



Fluorescence spectra of PFP



RSDs of migration times, peak heights, and peak areas of PFP-labeled maltooligosaccharide standards were determined to be 1.51–1.54 %, 2.24–3.10 %, and 2.78–7.43 %, respectively.
LODs were determined to be 31–38 nM (S/N = 3, n = 5).

References

[1] D. Smolkova, R. Cmelik, J. Lavicka, Trends Anal. Chem. 2023, 163, 117068

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[4] J. Schlecht, A. Stolz, A. Hofmann, L. Gerstung, C. Neusüß, Anal. Chem. 2021, 93, 14593–14598

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Conclusions

- **The hydrazide group enables efficient labeling via hydrazone formation chemistry, eliminating the need for a reduction step.**
- **The positive charge of the tags makes them ideal for both electrophoretic separation and MS detection in positive ion mode.**
- **With fluorescence excitation maxima in the range of 230-380 nm, these labels are well-suited also for LIF/LED-IF detection using commercially available solid-state laser or LED sources, enhancing detection sensitivity and quantitation limits.**
- **Electrophoretic analysis in a neutral-coated capillary achieved baseline separation of labeled oligosaccharides, with detection limits in the nanomolar concentration range.**
- **The optimized labeling and separation conditions have been successfully applied to *N*-linked glycan profiling of various glycoproteins, including therapeutic monoclonal antibodies.**