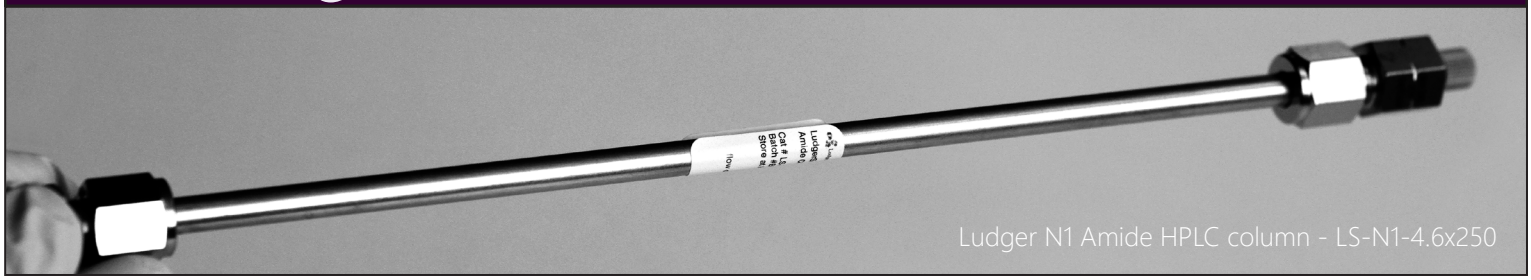
Ludger News

September/October 2019

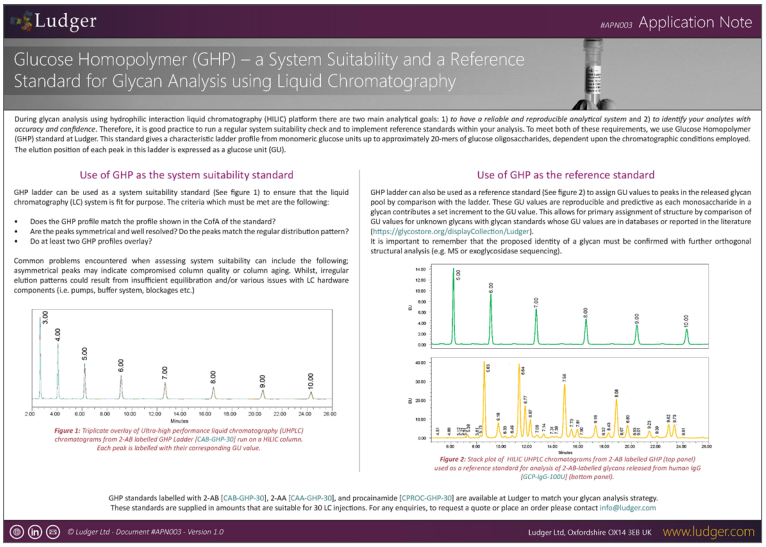


Application note for uses of Glucose Homopolymer ladder in Glycan Analysis

During glycan analysis using hydrophilic interaction liquid chromatography (HILIC) platform there are two main analytical goals: 1) to have a reliable and reproducible analytical system and 2) to identify your analytes with accuracy and confidence. Therefore, it is good practice to run a regular system suitability check and to implement reference standards within your analysis and at Ludger we use the Glucose Homopolymer (GHP) standard for our chromatographic glycan analysis.

Our new application note illustrates the uses of GHP standard as a system suitability standard and a reference standard to assist you with your chromatographic analysis.

We offer GHP standards labelled with 2-AB [CAB-GHP-30], 2-AA [CAA-GHP-30], and procainamide [CPROC-GHP-30]. To enquire or place an order please contact: info@ludger.com


Ludger #APN003 Application Note

Glucose Homopolymer (GHP) – a System Suitability and a Reference Standard for Glycan Analysis using Liquid Chromatography

During glycan analysis using hydrophilic interaction liquid chromatography (HILIC) platform there are two main analytical goals: 1) *to have a reliable and reproducible analytical system* and 2) *to identify your analytes with accuracy and confidence*. Therefore, it is good practice to run a regular system suitability check and to implement reference standards within your analysis. To meet both of these requirements, we use Glucose Homopolymer (GHP) standard at Ludger. This standard gives a characteristic ladder profile from monomeric glucose units up to approximately 20-mers of glucose oligosaccharides, dependent upon the chromatographic conditions employed. The elution position of each peak in this ladder is expressed as a glucose unit (GU).

Use of GHP as the system suitability standard

GHP ladder can be used as a system suitability standard (See figure 1) to ensure that the liquid chromatography (LC) system is fit for purpose. The criteria which must be met are the following:

- Does the GHP profile match the profile shown in the CofA of the standard?
- Are the peaks symmetrical and well resolved? Do the peaks match the regular distribution pattern?
- Do at least two GHP profiles overlay?

Common problems encountered when assessing system suitability can include the following; asymmetrical peaks may indicate compromised column quality or column aging. Whilst, irregular elution patterns could result from insufficient equilibration and/or various issues with LC hardware components (i.e. pumps, buffer system, blockages etc.)

Figure 1: Triplicate overlay of Ultra-high performance liquid chromatography (UHPLC) chromatograms from 2-AB labelled GHP Ladder [CAB-GHP-30] run on a HILIC column. Each peak is labelled with their corresponding GU value.

Use of GHP as the reference standard

GHP ladder can also be used as a reference standard (See figure 2) to assign GU values to peaks in the released glycan pool by comparison with the ladder. These GU values are reproducible and predictive as each monosaccharide in a glycan contributes a set increment to the GU value. This allows for primary assignment of structure by comparison of GU values for unknown glycans with glycan standards whose GU values are in databases or reported in the literature (https://glycostore.org/displayCollection/Ludger).

It is important to remember that the proposed identity of a glycan must be confirmed with further orthogonal structural analysis (e.g. MS or exoglycosidase sequencing).

Figure 2: Stack plot of HILIC UHPLC chromatograms from 2-AB labelled GHP (top panel) used as a reference standard for analysis of 2-AB-labelled glycans released from human IgG (GCP-IgG-100U) (bottom panel).

GHP standards labelled with 2-AB [CAB-GHP-30], 2-AA [CAA-GHP-30], and procainamide [CPROC-GHP-30] are available at Ludger to match your glycan analysis strategy. These standards are supplied in amounts that are suitable for 30 LC injections. For any enquiries, to request a quote or place an order please contact info@ludger.com

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For more information and to view the application note, please visit our webpage on glycan standards.

LudgerSep Buffers for WAX and HPLC Analysis of Glycans

LudgerSep buffers are produced to simplify the preparation of solvents for glycan analysis using either WAX columns or HPLC columns. The advantages of using our buffers include: ease of buffer preparation, consistence of pH for every batch purchased, there is no risk of contamination or out-of-specification problems that arise during glycan analysis when using LudgerSep buffers.

The table summarises the different applications; specifications including description, usage, storage and a link to the product guide to assist you with your glycosylation analysis workflow.

To view the pdf version of the table, please visit our Glycan HPLC and UHPLC columns and buffers webpage.

For any enquiries, to request a quote or place an order please contact: info@ludger.com

Ludger LudgerSep™ Buffers for WAX and HPLC Analysis of Glycans			
Catalogue #	LS-C-BUFFX4	LS-N-BUFFX40	LS-R-BPTX10
Buffer	**LudgerSep C Buffer x4 Concentrate**	**LudgerSep N Buffer x40 Concentrate**	**LudgerSep R BPT solvent x10 concentrate**
Application	Used in WAX (weak anion exchange) HPLC analysis of labeled glycans	For preparation of LS-N buffer (50 mM ammonium formate buffer, pH 4.4) used in amide or HILIC (hydrophilic interaction liquid chromatography) HPLC analysis of labeled glycans	For preparation of butylamine/ orthophosphoric acid/ tetrahydrofuran solvent (BPT) used in monosaccharide HPLC analysis.
Description	2.0M ammonium formate buffer/ solution pH9.0	50ml of x40 LS-N buffer (2.0M ammonium formate buffer/solution)	50 mL of x10 LS-BPT solvent
Usage	Dilute the whole contents of the bottle (50mL) with 150 mL HPLC grade water, then add 50 mL acetonitrile to make LS-C solvent (500 mM ammonium formate H 9, 20% acetonitrile v/v). The 50 mL of x4 buffer will make 250 mL of LS-C solvent.	Dilute with de-gassed HPLC grade water (use 1 part of x40 buffer to 39 parts of water) to make LS-N buffer (50 mM ammonium formate, pH 4.4). The 50 ml of x40 buffer will make 2 litres of LS-N buffer.	Dilute with de-gassed HPLC grade water use 1 bottle of LS-R-BPTX10 solvent to 450 mL of water. The 50 mL of x10 solvent will make 500 mL of BPT solvent.
Storage	Store unopened bottle below 25 °C. As with any HPLC solvent we recommend preparation of immediately before use.	Store unopened bottle below 25 °C. As with any HPLC solvent we recommend preparation of the solvent immediately before use.	Store unopened bottle at 4 °C. As with any HPLC solvent we recommend preparation of the solvent immediately before use.
Product Guide	ludger-ls-c-buffx4-guide	ludger-ls-n-buffx40-guide	ludger-ls-r-bptx10-guide

Summary of HPLC and UHPLC columns for glycan analysis

At Ludger we offer a range of HPLC and UHPLC columns to suit your specific chromatographic applications.

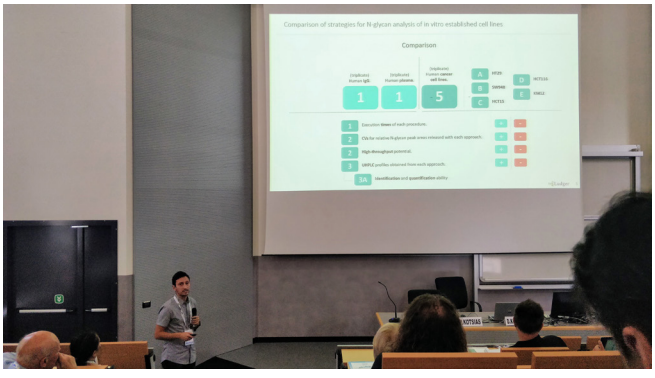
The table below summarises the different applications; column specifications including particle size, flow rates, column pressure, temperature and a link to the product guide to assist you with your chromatography analysis.

To view the pdf version of the table, please visit our Glycan HPLC and UHPLC columns and buffers webpage.
For any enquiries, technical advice or to request a quotation, please contact us at: info@ludger.com

Ludger LudgerSep™ HPLC and UHPLC Columns								
Catalogue #	LS-C2-4.6x50 and LS-C2-4.6x150	LS-C3-7.5x75	LS-N1-4.6x10 and LS-N1-4.6x250	LS-N2-4.6x150 and LS-N2-2.1x150	LS-R1-4.6x150	LS-R2-4.6x150	LS-UR2-2.1x50	LS-UR2-2.1x100
Column	**C2 anion exchange HPLC column**	**C3 anion exchange HPLC column**	**N1 amide HPLC column**	**N2 amide HPLC column**	**R1 HPLC column**	**R2 HPLC column**	**uR2 UHPLC column for monosaccharide analysis**	**uR2 DMB UHPLC column for sialic acid analysis**
Application	Separation of negatively charged glycans into neutral, mono, di, tri and tetra species.	Separation of negatively charged glycans into neutral, mono, di, tri and tetra species.	Separation of fluorophore labelled glycans according to size and shape.	Separation of fluorophore labelled glycans according to size and shape.	Separation of DMB labelled sialic acids on HPLCs	Monosaccharide analysis on HPLC systems	Monosaccharide analysis on UHPLC systems	Separation of DMB labelled sialic acids on UHPLCs
Description	Polystyrene particles with a macroporous polymeric anion exchange coating optimized for anion exchange chromatography. (1000 Angstrom pore size)	The C3 HPLC column contains macroporous (1000 Angstrom) anion exchange particles optimized for anion exchange chromatography of complex glycan mixtures.	LudgerSep N1 HPLC columns contain 5 um particles (80 Angstrom pore size) with a polymeric amide coating suitable for low resolution chromatography of complex glycan mixtures where UHPLC is not available.	LudgerSep N2 HPLC columns contain 3 um particles (80 Angstrom pore size) with a polymeric amide coating suitable for low resolution chromatography of complex glycan mixtures where UHPLC is not available and quicker gradients than N1 are required	The LudgerSep R1 HPLC column contains particles with an octadecylsilane coating optimized for hydrophobic chromatography. 120 Angstrom pore size	The LudgerSep R2 HPLC column contains particles with an octadecylsilane coating optimized for hydrophobic chromatography. It is optimized to handle efficient elution of the monosaccharides from the free dye peak. 175 angstrom pore size	The LudgerSep uR2 UHPLC column contains particles with an octadecylsilane coating optimized for hydrophobic chromatography. It is optimized to handle efficient elution of the monosaccharides from the free dye peak. 175 angstrom pore size	The LudgerSep uR2 UHPLC column contains particles with an octadecylsilane coating optimized for hydrophobic chromatography.
Particle size	8 um	10 um	5um	3um	3um	3um	1.9um	1.9um
Column Dimensions (width x length)	4.6mm x 50mm OR 4.6mm x 150 mm	7.5mm x 75mm	4.6mm x 10 mm OR 4.6mm x 250mm	4.6mm x 150mm OR 2.1mm x 150mm	4.6mm x 150mm	4.6mm x 150mm	2.1mm x 50mm	2.1mm x 100mm
Flow Rates	1mL/min	0.3 – 1.2mL/min	0.4 mL/min typical	0.4 mL/min typical	0.3 mL/min	0.3-2mL/min	0.4mL/min	0.25mL/min
Pressure	Max 3000 psi (207 bar)	Max 2175 psi (150 bar)	Max 2175 psi (150 bar)	Max 2900 psi (200 bar)	Max 5800 psi (400 bar)	Max 5800 psi (400 bar)	Max 18000 psi (1250 bar)	Max 18000 psi (1250 bar)
pH Range	1-14 pH stable	2-12	2-7.5	2-7.5	2-8	1-11	1-11	1-11
Temperature	10-80 °C	10-45 °C	30 °C typical (range 10-80 °C)	30 °C typical (range 10-50 °C)	60 °C max	60 °C max	60 °C max	60 °C max
Solvents	Acetonitrile, 20-500mM ammonium formate or ammonium acetate solvents	Acetonitrile, 20-500mM ammonium formate or ammonium acetate solvents	Acetonitrile and 50-250mM ammonium formate pH4.4	Acetonitrile and 50-250mM ammonium formate pH4.4	Acetonitrile + methanol mix	Solvent A: 0.2% butylamine/ 0.5% phosphoric acid/ 1% tetrahydrofuran in purified water. Solvent B: Acetonitrile	Solvent A: 0.2% butylamine/ 0.5% phosphoric acid/ 1% tetrahydrofuran in purified water. Solvent B: Acetonitrile	Acetonitrile + methanol mix
Companion Products	LudgerSep C Buffer x4 Concentrate LS-C-BUFFX4	LudgerSep C Buffer x4 Concentrate LS-C-BUFFX4	LudgerSep N Buffer x40 Concentrate LS-N-BUFFX40	LudgerSep N Buffer x40 Concentrate LS-N-BUFFX40	--	LudgerSep R BPT solvent x10 concentrate LS-R-BPTX10	LudgerSep R BPT solvent x10 concentrate LS-R-BPTX10	--
Product Guide	ludger-ls-c2-guide	ludger-ls-c3-guide	ludger-ls-n1-guide	ludger-ls-n2-guide	ludger-ls-r1-guide	ludger-ls-r2-guide	ludger-ls-ur2-guide	ludger-ls-ur2-dmb-guide

Ludger at Glyco25 2019

Maximilianos Kotsias (Scientist, Ludger) recently attended the 25th International Symposium on Glycoconjugates 2019 August 25th-31st in Milan, Italy. He presented a talk entitled “Advancements in glycoanalytical strategies for N- and O-glycan analysis from in vitro established cell lines”. Additionally, he took part in a round table discussion regarding the use of mass spectrometry for the analysis of glycans.



If you are interested in *N*- and O-glycan analysis workflows please visit our Glycan Analysis webpage and for any enquiries regarding our custom analytical services contact us at: info@ludger.com

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