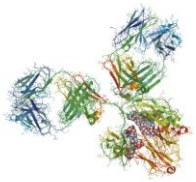

Product Guide for LudgerSep™ uR2 UHPLC Column for Sialic Acid Analysis

(Ludger Product Code: LS-UR2-2.1x100)

Ludger Document # LS-uR2-DMB-Guide-v1.1

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Analysis of sialic acids labeled with 1,2-diamino-4,5 methylenedioxybenzene.2HCl (DMB) using UHPLC.

Description

The LudgerSep™ uR2 UHPLC column contains particles with an endcapped octadecylsilane coating optimized for hydrophobic chromatography.

Particles

1.9 μm silica derivatized with octadecylsiliane coating. 175 Angstrom pore size.

Column Size

Cat #	Diameter x Length	Column Volume
LS-UR2-2.1x100	2.1 x 100 mm	0.34 ml

Column Tube

Stainless steel

Flow Rates

Typical flow rates = 0.1 – 0.6 mL/min.

Pressure

Pressure should not exceed 15000psi. Higher pressure applications result in reduced column lifespan.

pH Range

1 - 11

Temperature

Typical operating temperature = 30 ^{o}C, but increasing the temperature may improve resolution for some samples.
Maximum temperature range = 15 - 60 ^{o}C.

Solvents

An isocratic gradient of 7:9:84 methanol:acetonitrile:water is recommended for sialic acid analysis. Methanol is a volatile solvent and so it is recommended that the solvent is made up prior to use.
Avoid strong oxidants and anionic detergents.

Column Protection

Filter all solvents to 0.2 μm and degas using either helium sparging or vacuum degassing.
Filter all samples using a 0.2 μm filter membrane before loading onto the column.
Install a good quality in-line filter between the sample injector and the column.
Please call us for advice on the most suitable sample and in-line filters to use.
Long term storage of the column should be in solvent containing at least 30% acetonitrile.

Suitable Samples	DMB labeled sialic acids.
Sample	Filter samples to 0.2 μm and avoid exposure to light. For best resolution of chromatogram peaks use a full loop injection method with a sample loop of 5 µl volume or less. UHPLC peak resolution is highly dependent on minimized system volumes.
Preparation	Dissolve samples in 7:9:84 methanol:acetonitrile:water.
Sample Detection	Fluorescence. Excitation: 373 nm. Emission: 448 nm.
Handling:	Ensure that any glass, plasticware or solvents used are free of glycosidases and environmental carbohydrates. Use powder-free gloves for all sample handling procedures and avoid contamination with environmental carbohydrate.
Safety:	Please read the Material Safety Data Sheets (MSDS's) for all chemicals used. All processes involving labelling reagents should be performed using appropriate personal safety protection - eyeglasses, chemically resistant gloves (e.g. nitrile), etc. - and where appropriate in a laboratory fume cupboard

For research use only. Not for human or drug use

HPLC System Requirements

The LudgerSep™ uR2 column is designed for use with the latest generation of UHPLC instruments capable of withstanding high flow pressures and fast sample analyses. In order to take advantage of the high resolving power of sub 3 µm particle size containing columns, we recommend keeping sample injection volumes below 5 µL and minimising system void volumes. Ideally use full loop injection with a sample loop of less than 5 µL.Tubing should be narrow bore (about 0.13 mm diameter or less) and detector flow cell volumes should be 10 µl or less. Although an example chromatogram is shown in this guide, retention times will vary dependent on the UHPLC system used.

Installation of the Column

During column installation we recommend that :

- You should connect the LudgerSep™ uR2 column to your HPLC system using standard 0.13 mm ID tubing and zero dead volume connectors. Flow direction is indicated by an arrow on the column label.
- Keep the lengths of tubing between the injector to column and column to detector as short as possible to minimise sample dispersion effects.
- Ensure that all solvents are filtered prior to use.
- Before analysing any samples, the newly installed column should be conditioned using the protocol described below.

Preconditioning of the Newly Installed Column

The following preconditioning step is recommended prior to use of the column :

For sialic acid analysis flush the column at a flow rate of 0.25 mL/min with 7:9:84 methanol:acetonitrile:water.

Column Cleaning and Storage

After heavy use, your LudgerSep™ uR2 column may become contaminated with strongly adsorbed sample constituents that will lead to a loss in column performance.

Peptide or other components in the sample may cause retention times to shift over a period of time. To keep the column in good condition we recommend cleaning with a 30 min run of 50% sialic acid separation solvent, 50% acetonitrile at the end of each sample set.

The LudgerSep™ uR2 column should be stored in a low aqueous solvent. We recommend acetonitrile (minimum 30%) but other solvents such as isopropanol can be used.

Sample Preparation

Samples for injection onto the LudgerSep™uR2 column should be free of particulates. Particulates can be removed from samples using microcentrifuge filters with 0.2 μm pore size membranes.

Analysis of DMB-labeled Sialic Acids

For release of sialic acids from glycoproteins and labeling with 1,2-diamino-4,5-methylenedioxybenzene.2HCl (DMB), please follow the guide provided with the LudgerTag™ DMB Sialic Acid Labelling Kit, (Cat. No. LT-KDMB-A1)

The DMB labeled sialic acid reference panel (a component of the DMB sialic acid labelling kit) is an excellent sample to run on the LudgerSep™ uR2 column to ensure efficient performance of the column for sialic acid identification. An example DMB sialic acid reference panel chromatogram is shown below in Figure 1.

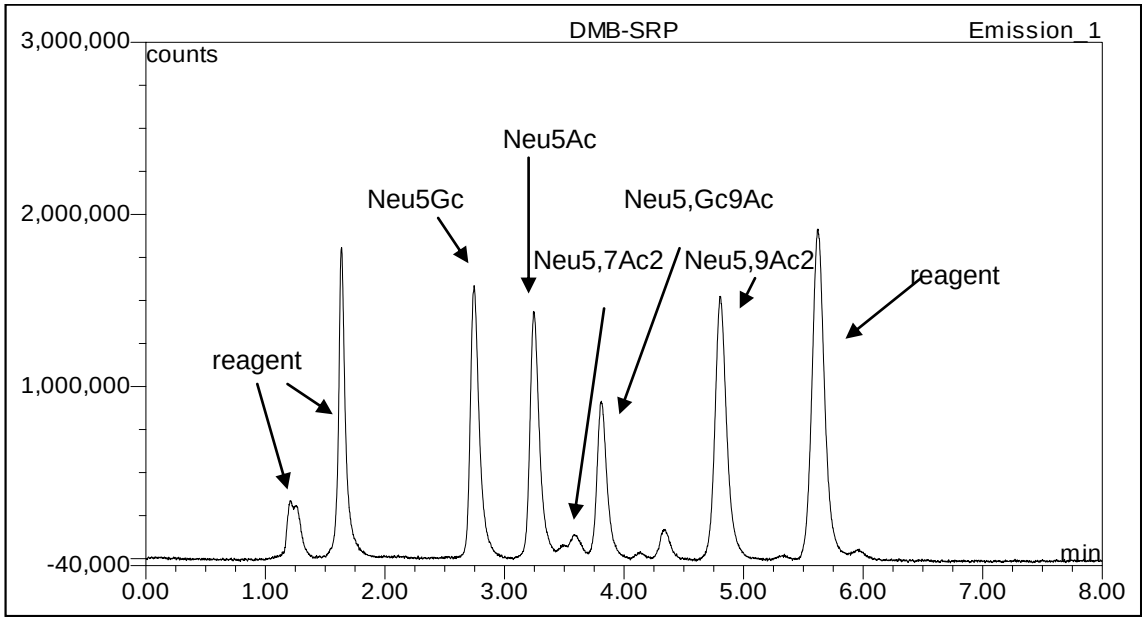

Figure 1: DMB Labeled Sialic Acid Reference Panel Run on the LudgerSep™ uR2 UHPLC column.

Solvents and Gradient

The glycan analysis gradients are based on the following solvents. We recommend using these as a guide to developing the most appropriate gradient for your HPLC system.

Separation Conditions:

An isocratic gradient at room temperature over a 10 min period is recommended.

Flow rate: 0.25 ml/min

Solvent: Methanol:Acetonitrile:Water (7:9:84)

Note: Store samples at -18°C in the dark after use (DMB is light sensitive)

Warranties and Liabilities

Ludger warrants that the above product conforms to the attached analytical documents. Should the product fail for reasons other than through misuse Ludger will, at its option, replace free of charge or refund the purchase price. This warranty is exclusive and Ludger makes no other warrants, expressed or implied, including any implied conditions or warranties of merchantability or fitness for any particular purpose.
Ludger shall not be liable for any incidental, consequential or contingent damages.

This product is intended for *in vitro* research only.

Document Revision Number

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Appendix 1 : Troubleshooting Guide

Analysis of DMB labeled sialic acids using the LudgerSep uR2 is a robust method. If problems do arise they can usually be corrected without difficulty. The following is a guide to the most likely problems, possible causes, and solutions.

A. Samples are not retained on the column or elute early.

1. The column may not be fully equilibrated. Ensure that the column is washed thoroughly in Solvent A before running samples.
2. The solvent has gone off. The chromatography is sensitive to accurate levels of each solvent component. High levels of methanol will result in earlier elution of the sialic acids.
3. The column is contaminated. Perform an overnight wash of the column in 50-70% acetonitrile at a low flow rate (about 0.1 mL/min).

B. Samples are retained and cannot be eluted from the column.

1. The HPLC solvent is not fresh. Methanol is a volatile organic solvent which evaporates from the running solvent over time. Check that the solvent has not been stored on the HPLC for longer than two weeks, and that environmental conditions that may cause increased evaporation have been minimised eg avoid direct sunlight on the solvents, air flow over the solvent should be minimized, the temperature of the laboratory should be kept constant at about 21 degrees celcius.

C. The sialic acids have different retention times to a previous column or the example chromatogram.

The chromatogram in this guide is provided as an example only. Peak width, resolution and retention are highly dependent on the UHPLC system setup in your laboratory.

1. Solvent variability. Check that solvents are prepared methodically for each batch.

2. Column variability. Whilst every effort is made to ensure each column resin batch is the same, some variations may occasionally occur. A certain amount of variation is acceptable, but if separation performance is lower than expected, please contact Ludger and we will assist to remedy the situation.