News - May 2014

Automation News

Following our ongoing collaboration with Hamilton Robotics we have worked successfully together to release the following Application Note:

N-glycan characterisation of biopharmaceuticals: simplifying sample preparation with the Microlab STARlet

This Application Note describes the adaptation of the Ludger VP-T1 system (i.e. N-glycan labelling, using 2-picoline borane, and SPE clean-up) to a Hamilton Microlab STARlet liquid handling robot. This method gives excellent repeatability and intermediate precision (Figure 1). The system is simple to operate, scalable (up to 96 samples can be processed simultaneously) and is safer to use than traditional sodium cyanoborohydride methods.

The work flow is a three-step process and the total time taken is 4.5 hours:

1. Labelling of glycan samples with 2AB or 2AA using LudgerTag VP kits
2. SPE sample clean-up using LudgerClean T1 cartridges
3. Preparation of samples for HPLC/UPLC analysis

For a copy of the Application Note please contact us at info@ludger.com

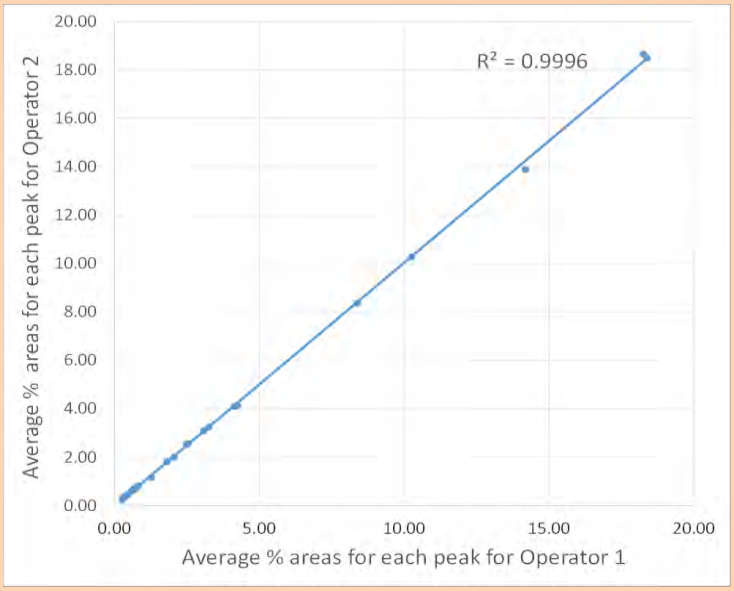

Figure 1: The scatter chart shows repeatability (intermediate precision) data: the data sets for 32 samples analysed by Operator 1, day 1 and 32 samples analysed by Operator 2, day 2 are compared

Publication

Along with colleagues at Leiden University and VU University in the Netherlands we are delighted to have had a paper published in Analytical Biochemistry. The paper is entitled:

Improved nonreductive O-glycan release by hydrazinolysis with ethylenediaminetetraacetic acid addition

Anal. Biochem **45** 3 (2014) 29-37

In the paper, Radoslaw Kozak *et al* describe how EDTA can be used during hydrazinolysis to reduce 'peeling' during O glycan release.

This improved method has a reduced number of sample handling steps, compared with the previously published protocol, which makes the process much quicker and helps to prevent sample losses when working with small amounts of materials.

If you would like more information please contact us at info@ludger.com

More Tools For Quantitation

We have now added to our BioQuant™ range by producing quantitative standards using **chitotriose**, a linear tri-N-acetylglucosamine glycan. This is available as an unlabelled glycan or labelled with 2-AB and 2-AA. The amount of chitotriose dispensed per vial has been determined by quantitative Nuclear Magnetic Resonance (qNMR) of the bulk chitotriose stock.

Use as an internal standard

Our unlabelled chitotriose standard (**Cat: # BQ-CHITOTRIOSE-01**) can be used as an internal standard to quantify glycans in your sample. To do this, add a known amount of the unlabelled chitotriose to your unlabelled glycan sample. The mixture is then fluorescently labelled, cleaned up and run on HPLC/UHPLC. The unknown quantity of the glycan is calculated by comparison of the chitotriose and glycan peak areas (Figure 2). The advantage of this method is that any sample loss occurring during the labelling and clean up stages will be the same for the sample and the chitotriose, thereby removing this as a source of error. The results obtained when running this method compare favourably with monosaccharide testing.

Use as external standards

The 2-AB and 2-AA labelled chitotriose standards (**Cat: # BQ-CAB-CHI-01** and **Cat: # BQ-CAA-CHI-01** respectively) can be used as external standards to quantify glycans; these standards can be run directly on the HPLC/UHPLC. Again, the quantity of glycans in your sample is calculated by comparison of the peak areas for chitotriose (known amount) and your sample. This method is useful when quantification of already labelled glycans is required. They are also useful when quantification of several glycan samples is required in the same run, as the labelled chitotriose standard can be used as external standard for all the samples.

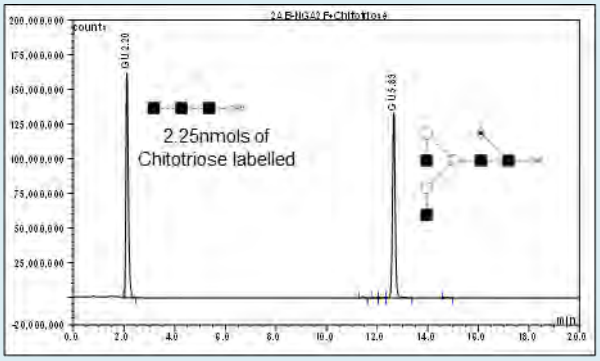

Figure 2: *HILIC UHPLC column chromatogram of NGA2F (Cat# CN-NGA2F-20U) and Chitotriose glycans (Cat: # BQ-CHITOTRIOSE-01) which have been labelled with 2AB.*

If you would like a quotation for either of these products please contact us via e-mail: info@ludger.com