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

Preparing N-glycan samples that have been released from therapeutic glycoproteins (biopharmaceuticals) for analysis can be challenging, as the fluorescent labelling and clean-up steps are time consuming. Laboratories require a system that is simple to operate, scalable and repeatable.

- *Simple to operate*
- *Scalable sample processing for up to 96 samples*
- *Improving the repeatability and reliability of the method*

INTRODUCTION

N-linked glycan characterisation is now accepted as a requirement for biopharmaceutical characterisation. Optimal glycosylation is critical for biopharmaceuticals, as glycans can influence their yield, immunogenicity and efficacy, which impact the costs and success of the treatment. To begin the process of characterisation, N-glycans are released from biopharmaceuticals using an endoglycosidase, PNGase F. Released N-glycans are then processed to allow their analysis by fluorescent HPLC/UPLC. The N-glycan sample labelling and clean-up processes have been adapted to the Microlab STARlet without compromising repeatability and intermediate precision. 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.
This application note describes the automated labelling and clean-up of released N-glycans and their preparation for HPLC/UPLC analysis using the Hamilton Compressed O-Ring Expansion (CO-RE) grip technology and the integrated Microlab Crystal Clear Manifold & Vacuum Station (CVS).

METHOD DESCRIPTION

The work flow is a three-step process:

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**

Once released from glycoproteins using a PNGase F in-solution digest, the dried down N-glycan samples in a 96 well PCR plate are transferred to the Hamilton STARlet. The N-glycans are labelled with 2-aminobenzamide (2AB) or 2-aminobenzoic acid (2AA) using LudgerTag VP kits set up on the Microlab STARlet deck. The kits process 96 samples and toxic sodium cyanoborohydride reductant has been replaced with a safer alternative, 2-picoline borane (2PB). Off deck incubation to allow sample labelling is performed in 1 hour. Samples are then cleaned up to remove excess labelling reagents using LudgerClean T1 cartridges with the on deck CVS. Clean-up of 96 samples is achieved in 2.5 hours. Finally, acetonitrile is mixed with the samples and the plate can then be placed directly into the HPLC/UPLC.

Ludger

HAMILTON

RELIABLE SAMPLE PROCESSING FOR N-GLYCAN ANALYSIS

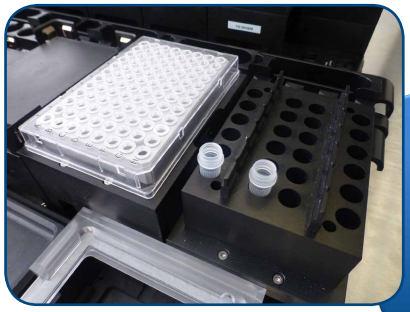
The Microlab STARlet automates the Ludger N-glycan sample labelling and clean-up methods so that once ready, plates of samples can be directly placed into the HPLC/UPLC system for analysis.

SYSTEM DESCRIPTION

The system is a Microlab STARlet liquid handling workstation with 8 independent pipetting channels. The deck is manually loaded with carriers containing tips, reagents, the filter plate and the PCR plate containing samples. The CVS is integrated on the deck. The plate movements as well as the loading and unloading of the vacuum manifold during the process are performed by the CO-RE Gripper.

WORKFLOW

GLYCAN SAMPLES



STAGE 1

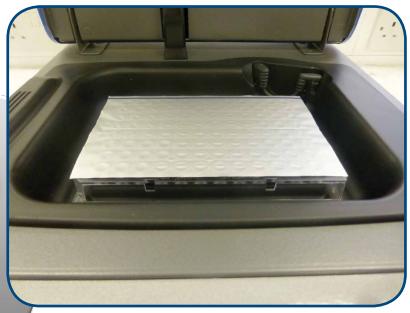
Label with 2AA or 2AB using LudgerTag VP kits

0.5 hours

OFF ROBOT

Seal plate and incubate at 65 °C then cool and return to robot

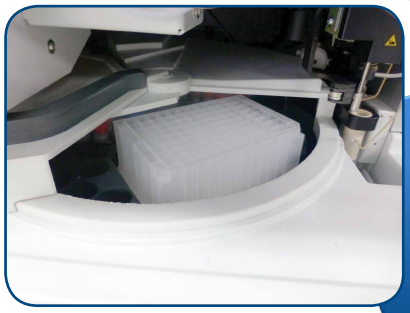
1 hour



STAGE 2

Sample clean-up using LudgerClean T1 cartridges

2.5 hours



STAGE 3

Preparation for HPLC/ UPLC analysis: acetonitrile is mixed with samples

0.5 hours

KIT DESCRIPTION

A LudgerTag 2-aminobenzamide (2-AB) VP kit was used for labelling up to 96 glycan samples, Cat # LT-KAB-VP96.

A kit containing 2-aminobenzoic acid (2AA) is also available, Cat # LT-KAA-VP96.

The kits are designed for use with samples in a 96 well microplate and the labelling process utilises 2-picoline borane as a reductant.

LudgerClean T1 cartridges were used for clean-up of 2AB labelled samples prior to analysis, Cat # LC-T1-A6. Unit size: 6 cartridges per pack.

PUT PLATE DIRECTLY INTO HPLC/UHPLC

APPLICATION SOFTWARE

The method was created using Hamilton's open and flexible Microlab VENUS software. A GUI leads the user through the instrument set-up process. Further application relevant parameters (e.g. vacuum settings, filtration times) can easily be adjusted by the user.

VALIDATION

The methods for labelling and clean-up of glycan samples using LudgerTag VP kits and LudgerClean T1 cartridges on the Microlab STARlet have been validated according to ICH Q2(R1) guidelines.
A number of validation characteristics were investigated as follows:

- Specificity
- Accuracy
- Repeatability (intra-assay precision)
- Repeatability (intermediate precision)
- Robustness

Throughput: The Microlab STARlet can process 96 samples in a PCR plate in 3.5 hours. The total time taken for labelling and clean-up is 4.5 hours

TECHNOLOGY

Hamilton´s CO-RE grip technology enables consistent tip and tool pick up, enhancing accuracy and precision of pipetting and the movement of labware on deck.
This enables automated loading and unloading of the vacuum manifold during the process. The integrated vacuum manifold allows clean-up of labelled glycan samples using LudgerClean T1 cartridges to be fully automated.
The level of vacuum can be finely controlled.

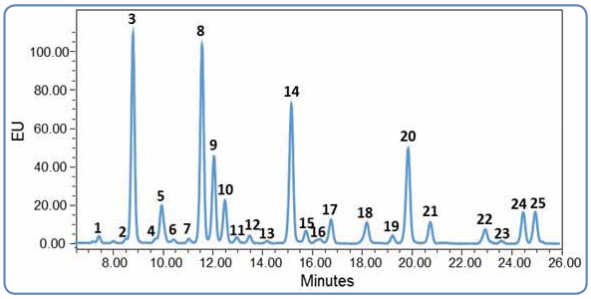


Fig. 1: A representative fluorescent UPLC chromatogram of the 2AB labelled N-glycan profile from human IgG. The main peaks that are present are labelled from 1 to 25

RESULTS

32 N-glycan samples from human IgG were processed by operator 1 on day 1 and 32 samples were processed by operator 2 on day 2 using the automated protocol on the Microlab STARlet. Analysis of these samples was performed by UPLC and a typical chromatogram of the N-glycan profile of human IgG is shown (fig. 1).

Repeatability (intra-assay precision) data was obtained from the results of the analysis of 32 samples from operator 1 on day 1 (fig. 2). The CVs for the average relative % peak area are <5% for glycan peaks with an average relative % area >2.5% (which includes 20 of the glycan peaks).

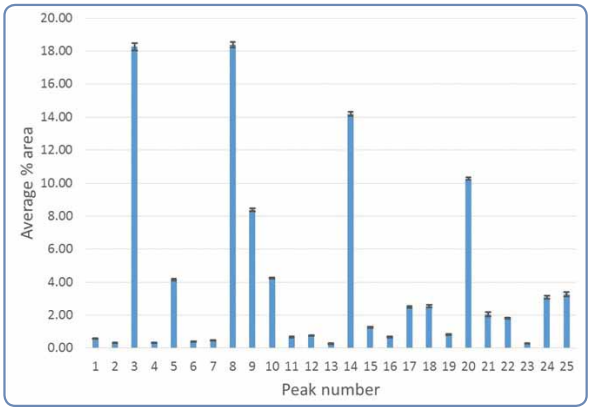


Fig. 2: The graph shows the average relative % peak areas for the 25 N-glycans released from human IgG sample by Operator 1. The error bars on each peak show the standard deviation from 32 samples analysed by Operator 1

Repeatability (intermediate precision) data was obtained from the results of the analysis of 32 samples from operator 1 on day 1 and 32 samples from operator 2 on day 2 (fig. 3). On the comparison of the two data sets, for peaks with a relative % area >1.5% (which includes 21 of the glycan peaks), the % variation is <5%. The R^2 value is close to 1 showing a good correlation and low variation between the two data sets.

DISCUSSION

Our hands-on experience has shown that teaming the Ludger glycan labelling and clean-up technology with the Microlab STARlet has improved laboratory efficiency without compromising reliability of data.

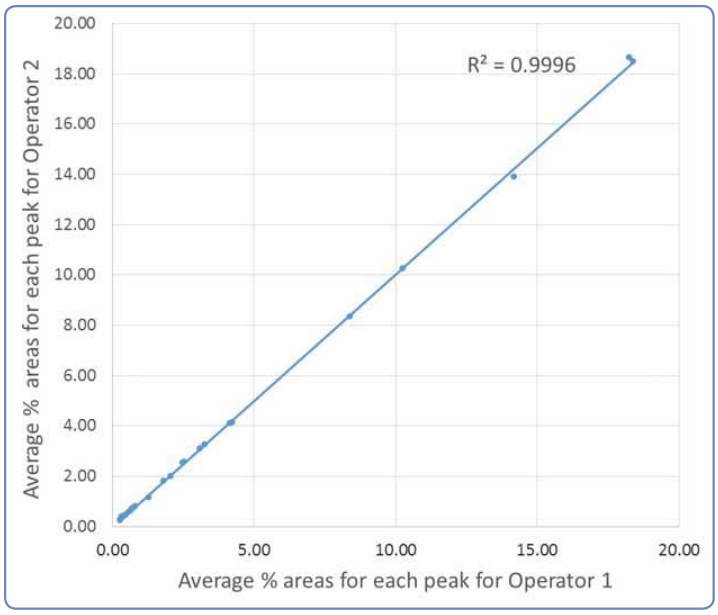


Fig. 3: The scatter chart shows Repeatability (Intermediate Precision) data: the data sets for 32 samples analysed by Operator 1, day 1 and 32 samples analysed by Operator 2, day 2 are compared.

System Requirements

Microlab STARlet
8 x 1 µl channels
CO-RE Gripper
Crystal Clear Manifold & Vacuum Station (with the short, chemical resistant manifold top)
Carriers for tubes, plates, 96 well PCR plate, reagents and disposable tips

System Dimensions

Width: 1124mm, Height: 903mm, Depth: 795mm

Reagents and accessories	Part number / Provider
LudgerTag 2AB labelling kit	LT-KAB-VP96 (Ludger)
LudgerTag 2AA labelling kit	LT-KAA-VP96 (Ludger)
LudgerClean T1 cartridges (6 per pack)	LC-T1-A6 (Ludger)
Cartridge Holder	LP-HOLDER-96 (Ludger)
Plug pack (12 strips of 8 plugs)	LP-PLUG-96 (Ludger)
Collection Plates (pack of 5)	LP-COLLPLATE-2ML-96 (Ludger)
Collection Plate Lids (pack of 5)	LP-COLLPLATE-LID-96 (Ludger)
Screw Cap Polypropylene Microtubes, 2ml	
96 well microplate (100 - 300 µl volume)	

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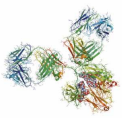

Ludger

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