Fully automated sample preparation with ultrafast N-glycosylation analysis of therapeutic antibodies

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There is a growing demand in the biopharmaceutical industry for high throughput, large scale N-glycosylation profiling of therapeutic antibodies in all phases of product development, but especially during clone selection where hundreds of samples must be analyzed in a short period of time. SCIEX has recently developed a magnetic bead based protocol for N-glycosylation analysis of glycoproteins to replace centrifugation and vacuum-centrifugation steps the currently used. Glycan release, fluorophore labeling and clean-up were all optimized resulting in a <4 hours magnetic bead mediated process with excellent yield and high reproducibility. This Technical Information Bulletin demonstrates an extension of this work by fully automating all steps of the optimized magnetic bead based protocol. Optimization includes endoglycosidase digestion through rapid fluorophore labeling and clean-up using high throughput 96-well plate sample processing in an automated liquid handling. Capillary Electrophoresis – Laser Induced Fluorescence (CE-LIF) analysis of the fluorophore labeled glycans was also optimized to enable rapid (<3 min) separations in order to accommodate high throughput of the automated sample preparation process.

Liquid phase-based glycanalytical methods generally require labor intensive and time consuming sample preparation and derivatization steps including glycan release, purification, fluorophore labeling and pre-concentration prior to analysis. In addition, current protocols include numerous centrifugation and vacuum-centrifugation steps that make full automation of the process by liquid handling robots difficult and expensive. Utilizing a novel magnetic bead mediated sample preparation protocol, a large number of samples can be processed in less than 4 hours in a 96 well plate format without the requirement centrifugation or vacuum-centrifugation steps. This new protocol has been tested using a Biomek FX\textsuperscript{\textregistered} Laboratory Automation Workstation for preparation of immunoglobulin G samples. Ultrafast analysis of the resulting fluorophore labeled glycans was accomplished in less than 3 minutes per sample using the PA 800 Plus capillary electrophoresis system configured with laser induced fluorescent detection.

Figure 1: The PA 800 Plus Pharmaceutical Analysis System with laser induced fluorescence detection (CE-LIF).

Experimental Setup

Automated sample preparation was performed on a Biomek FX\textsuperscript{\textregistered} Laboratory Automation Workstation (Beckman Coulter, Brea) (Figure 2) which was set up with 96 well plate holders, a magnetic stand, 1000 µl and 25 µl pipette tips, a quarter reservoir, along with sample and reagent vials. The quarter reservoir contained acetonitrile (Sigma Aldrich, MO) and the Agencourt CleanSeq magnetic beads (Beckman Coulter). The reagent vials contained reagents for the PNGase F digestion (Prozyme, Hayward, CA). 8-aminopyrene-1,3,6-trisulfonate (APTS) (Beckman Coulter, sold through SCIEX, Brea, CA) in 20% acetic acid and 1 M sodium-cyanoborohydride (in THF) (Beckman Coulter, sold through SCIEX). To reduce evaporation induced volume loss, a pipette box lid was used to cover the quarter reservoirs. The glycoprotein samples were incubated in a Biomek vortex heater block. For adequate re-suspension, an extra empty sample plate was applied under the actual sample plate, in which case the magnets were positioned under the sample tubes, rather that of at the side. In this configuration the magnet could pull down the magnetic beads to the bottom of the vials and with fast aspiration/dispensing the beads could be easily re-suspended.
Methods

The individual steps of the manual approach of the magnetic bead mediated sample preparation protocol were recently published by Varadi et al. [1]. The entire workflow is shown in Figure 3. Enzymatic digestion using PNGase F was performed at 50°C for 1 hour followed by glycan capture on the magnetic beads in 87.5% acetonitrile medium [2]. APTS labeling of the bound carbohydrates was initiated in situ on the beads by the addition of sodium cyanoborohydride followed by incubation at 37°C for 2 hours. The fluorophore labeled glycans were eluted from the beads by the addition of 25 µl of HPLC grade water and were analyzed by CE-LIF analysis using a PA 800 Plus equipped with LIF detection (488 nm excitation, 520 nm emission) (Beckman Coulter, sold through SCIEX, Brea, CA). For the separation, 20 cm effective length NCHO capillaries (Beckman Coulter) were used (30 cm total length, 50 µm ID) with 25 mM lithium acetate (pH 4.75) background electrolyte containing 1 % PEO (900,000, Sigma-Aldrich). The applied voltage was 30 kV and the separation temperature was 20°C. The samples were pressure injected by 3 psi for 6 seconds. The entire liquid handling protocol was programmed using the Biomek Software version 4.0 (Figure 4). The CE-LIF data were acquired and analyzed by the 32 Karat™ software package (Beckman Coulter, sold through SCIEX, Brea, CA).
Figure 3: Magnetic bead mediated sample preparation flowchart for N-glycosylation analysis of therapeutic antibodies by CE-LIF.

Results and Discussion

In this work, our earlier published magnetic bead mediated glycan release, APTS-labeling and sample clean-up protocol [1] was applied to a 96 well plate format utilizing the Biomek FX® Laboratory Automation Workstation (Figure 2). Results illustrated that by applying full automation, the process could be shortened to < 3.5 hours of processing time with excellent yield and high reproducibility. Programming of the liquid handling platform was simple and the system was flexible and robust, capable of handling a large number of samples. The Laboratory Automation Workstation offered a fast sample preparation option, reduced flow-induced shear strain on native biological sample matrices and minimized contamination risks.

Figure 4: The liquid handling protocol, programmed by the Biomek Software ver 4.

Due to the large amount of deck space available in the liquid handling system, buffer preparation for CE-LIF analysis was also performed using the Biomek FX to automate the solubilization step of the separation polymer matrix as defined in the Methods section.

The resulting fluorophore labeled glycans were analyzed using CE-LIF optimized for rapid separation to accommodate the high throughput of the fully automated sample preparation process. APTS labeled IgG glycan were injected consecutively from the 96-well plate taken directly from the liquid handler (Figure 5). Please note that baseline separation of the major IgG glycans was obtained in less than 3 minutes. This separation method can...
be readily applied to large scale processes like clone selection where rapid analysis of hundreds of samples is crucial.

In summary, the PA 800 Plus capillary electrophoresis system in combination with automated liquid handling used in these work was capable of precise and robust high throughput sample preparation for rapid N-glycosylation analysis of IgG molecules.

References


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