

High-Throughput Glycan Screening to Remove Biopharmaceutical Development Bottlenecks

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Introduction

Development of biopharmaceuticals comprise of many integrated steps, beginning with research & discovery, and optimally ending with a commercial therapeutic molecule. Essential to this development is screening large numbers of clones and cell culture expression conditions to find those combinations that express candidate proteins with the greatest likelihood of advancing into the clinic. Screening generally focuses on qualitative analyses of a cell line's protein titer, glycan expression profile, purity, charge heterogeneity, and tendency to aggregate. To ensure efficiency in cell line development, fast access to critical product information is important for in-process course correction. Current analytical strategies tend to create bottlenecks because they provide this product information in weeks rather than the same-day results necessary for making in-process adjustments.

This note describes the SCIEX C100HT Biologics Analyzer, an integrated, multiplexed capillary electrophoresis system capable of processing as many as five 96 well plates of protein samples in one day and qualitatively characterizing the glycan populations associated with each sample. The system includes automated sample glycan identification and intuitive data output, allowing for highly visual screening and easy determination of inter-sample quantitative differences. The analysis of a combination of synthetic glycans, IgG 1 kappa antibody derived from cell culture supernatant purified by protein A, and MAK33 purified monoclonal antibody drug product demonstrates system function and reproducibility.



Figure 1. SCIEX C100HT Biologics Analyzer

Key Features:

- Reproducible, high-throughput screening of glycans from cell cultures – as many as five 96 well plates of samples in one day
- Easy system set-up
- Fast, automation-compatible sample preparation
- Seamless data generation and integration for entire plates
- Intuitive pass/fail criteria based on customizable plate profiles
- Portable separation principle that can be used in all phases of biopharmaceutical development and quality control, minimizing the need for lengthy bridging studies

Overview

Analytical System

The SCIEX C100HT Biologics Analyzer is a high-throughput screening platform designed for glycan profiling of protein biologics. It applies 12 channel capillary electrophoresis (CE) separation with LED-IF excitation at 465 nm and emission at 540 nm, which allows for the detection of 1-aminopyrene-3,6,8-trisulfonate (APTS) labeled N-glycans released from monoclonal antibodies (mAbs) and similar protein biologics. The 12 capillaries are incorporated into a pre-filled, ready-to-use cartridge with monitoring technology to track cartridge life, calibration status, and remaining runs. The C100HT Glycan Screening Kit includes reagents, cartridge, and pre-filled, sealed buffer trays.

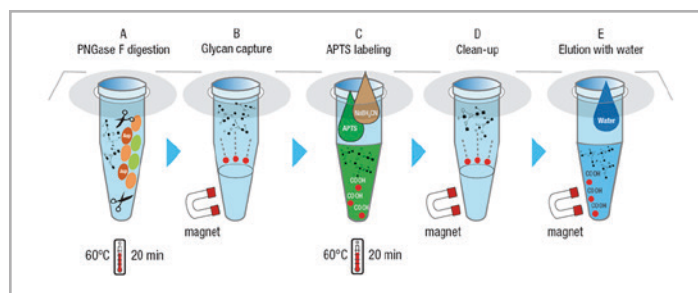


Figure 2. Glycan sample preparation for C100HT analysis.

Glycan Sample Preparation

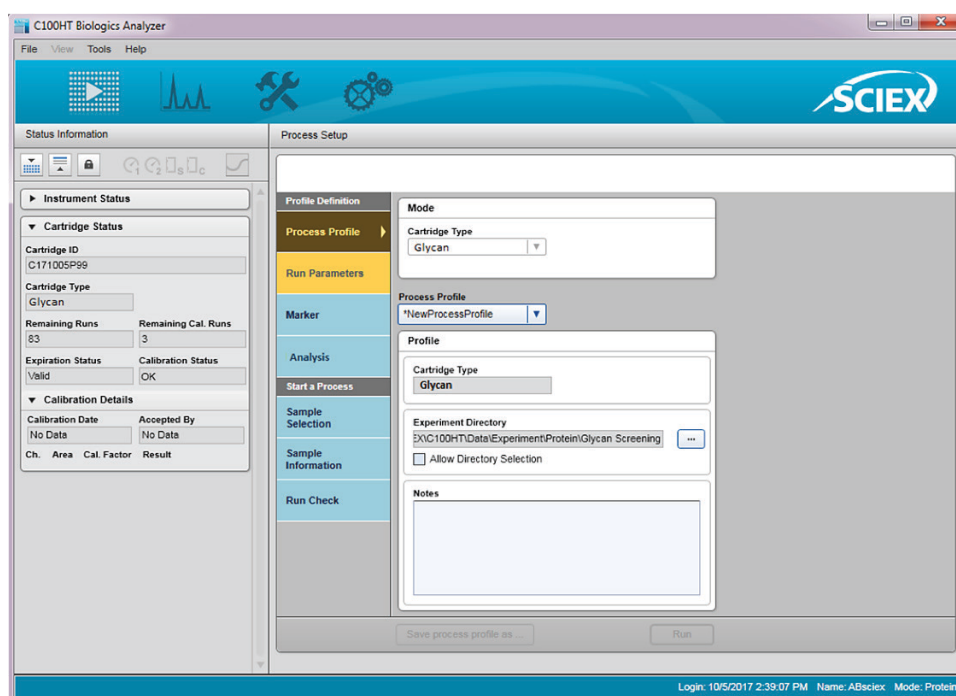
The C100HT analyzer uses the award winning SCIEX glycan separation workflow. Up to 96 samples can be prepared in less than two hours using automation (Beckman Coulter Biomek i5) or less than three hours performed manually. Sample preparation begins with a therapeutic protein sourced from cell culture, clone selection, or another in-process step. Using PNGase F, glycans are released from the protein, captured, and then labeled with APTS. Excess APTS is removed from the labeled glycans using a magnetic-bead-mediated series of washes (Figure 2).

Plate Loading and Separation

The C100HT Biologics Analyzer is intuitive and easy to use. It only takes minutes to set up the instrument, starting with insertion of the pre-filled buffer tray, sample plate, and pre-loaded separation cartridge. Then, the user selects the appropriate pre-configured process profile (Figure 3). Next, in the Sample Selection screen, the user inputs the sample information or other identifiers manually or through import of a .xls file. The analysis can be started by clicking the Run button.

Once analysis begins, a progress bar displays remaining separation time for the complete plate. Analysis of a full 96 well plate typically takes 170 minutes. Users can visualize the separation in real time and toggle between electropherogram view (Figure 4A) and gel view (Figure 4B).

Figure 3. Cartridge status and process setup in the C100HT software.



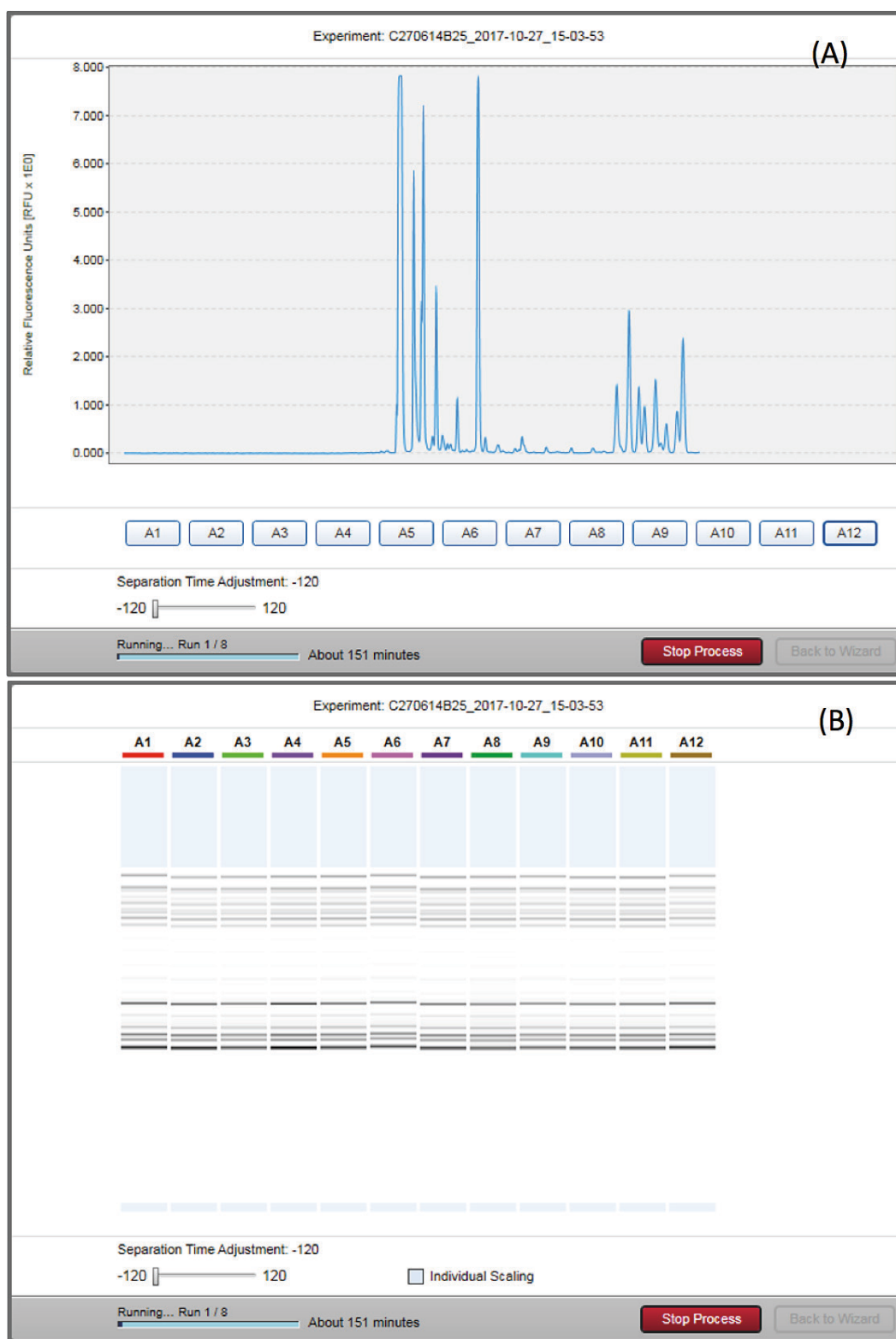


Figure 4. (A) Electropherogram view and (B) gel view in the C100HT software.

Upon completion of the electrophoretic separation, the data is automatically integrated and processed in the DataReviewer

software, which self-launches to display the results. This virtually eliminates the need for tedious user-driven data analysis.

Data Review

The DataReviewer software is an intuitive analysis interface that allows for easy visualization of results based on a pre-defined qualification profile. In the qualification profile, for each glycan of interest, the user can define the corrected area, % threshold and the logic (corrected area % greater than or equal to; corrected area % less than or equal to) that is desired. If a glycan is not to be qualified, it can be disabled by clicking on the appropriate radio button. Once the qualification profile is applied to the data, DataReviewer dynamically labels each well on the 96 well plate graphic with either a green or red color for a pass or fail, respectively (Figure 5). A different qualification profile can be applied to each individual sample, to multiple wells, or the entire plate. When a well is associated to a currently opened qualification profile, a purple star is placed next to the well graphic.

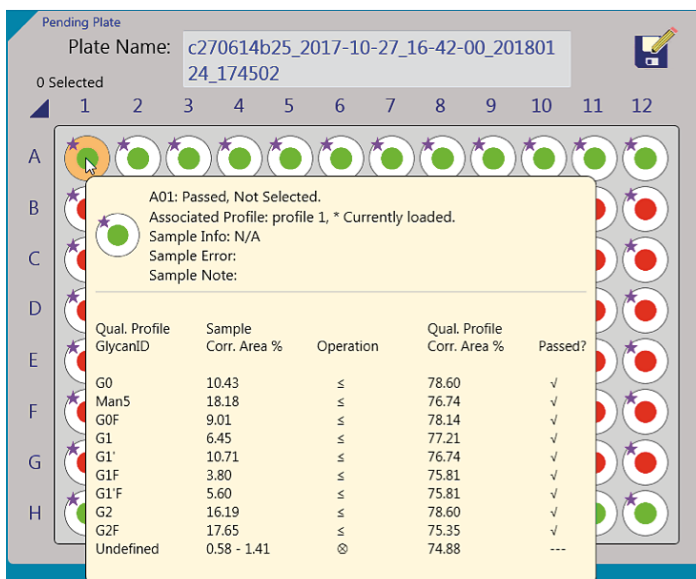


Figure 5. DataReviewer software displaying the glycans identified in well A1 with corresponding qualification profile and pass/fail result.

For rapid screening of a plate, the user can access the corrected area % for each well by placing the mouse over the desired well graphic, where a screen will display the corrected area % of glycans and passing or failing criteria.

Reports for each well or the entire plate can be created in a .pdf format.

Materials and Methods

To demonstrate C100HT Biologics Analyzer workflow and performance, a plate containing a synthetic glycan panel, protein A purified IgG1 kappa antibody, and MAK33 purified monoclonal antibody drug product was prepared and analyzed.

Sample Preparation

MAK33 is a commercial monoclonal antibody drug product obtained from Roche Diagnostics (Indianapolis, IN). PNGase F was obtained from New England Biolabs (Boston, MA). Acetonitrile was obtained from Burdick-Jackson. A 1M solution of sodium cyanoborohydride in THF was obtained from Sigma-Aldrich (St Louis, MO). Reagents used to prepare the denaturation solution, digestion solution, and labeling solution were provided in the chemistry kit (SCIEX, PN C13787). A glycan panel was prepared by labeling and mixing 9 synthetic species.

200 µl of the magnetic beads mixture were dispensed into each well of a 96 well plate. After magnetic capture of the beads and removal of the storage solution, 100 µg of sample was added to the beads as follows:

Rows A, H – 9 glycan panel used as system suitability standard

Rows B, D, and F – Commercial purified antibody

Rows C, E, and G – IgG 1 K antibody (protein A captured from cell culture material)

The antibodies were denatured for 8 minutes at 60° C using the denaturing solution master mix and digested for 20 minutes at 60° C using PNGase F. The resulting glycans were captured using a magnetic-bead-mediated process. The glycans were then labeled with APTS for 20 minutes at 60° C. Excess label was removed using a bead-mediated process repeated 3 times. The labeled N-linked glycans were eluted from the beads by adding 50 µL of water.

At this point, the samples were analyzed by electrophoretic separation using the C100HT Biologics Analyzer.

Results and Discussion

Data Review

Upon completion of electrophoretic separation by the C100HT Biologics Analyzer, the data was automatically imported into the DataReviewer software. The DataReviewer software took just over 17 minutes to analyze the full 96 well plate. DataReviewer software reported out corrected area %, GU values, glycan identities, and relative migration times. Figure 6 illustrates the

full 96 well plate results. Each well of Rows A and H contained the 9 glycan panel, rows B, D and F contained commercial purified antibody, and rows C, E, and G contained the IgG 1 K antibody (protein A captured from cell culture material).

For demonstration purposes, a qualification profile with pass criteria only applicable for the 9 glycan panel was chosen. The software dynamically responded to the profile settings by labeling the wells green if samples met the qualification profile or red if they failed to meet the requirements of the qualification profile.

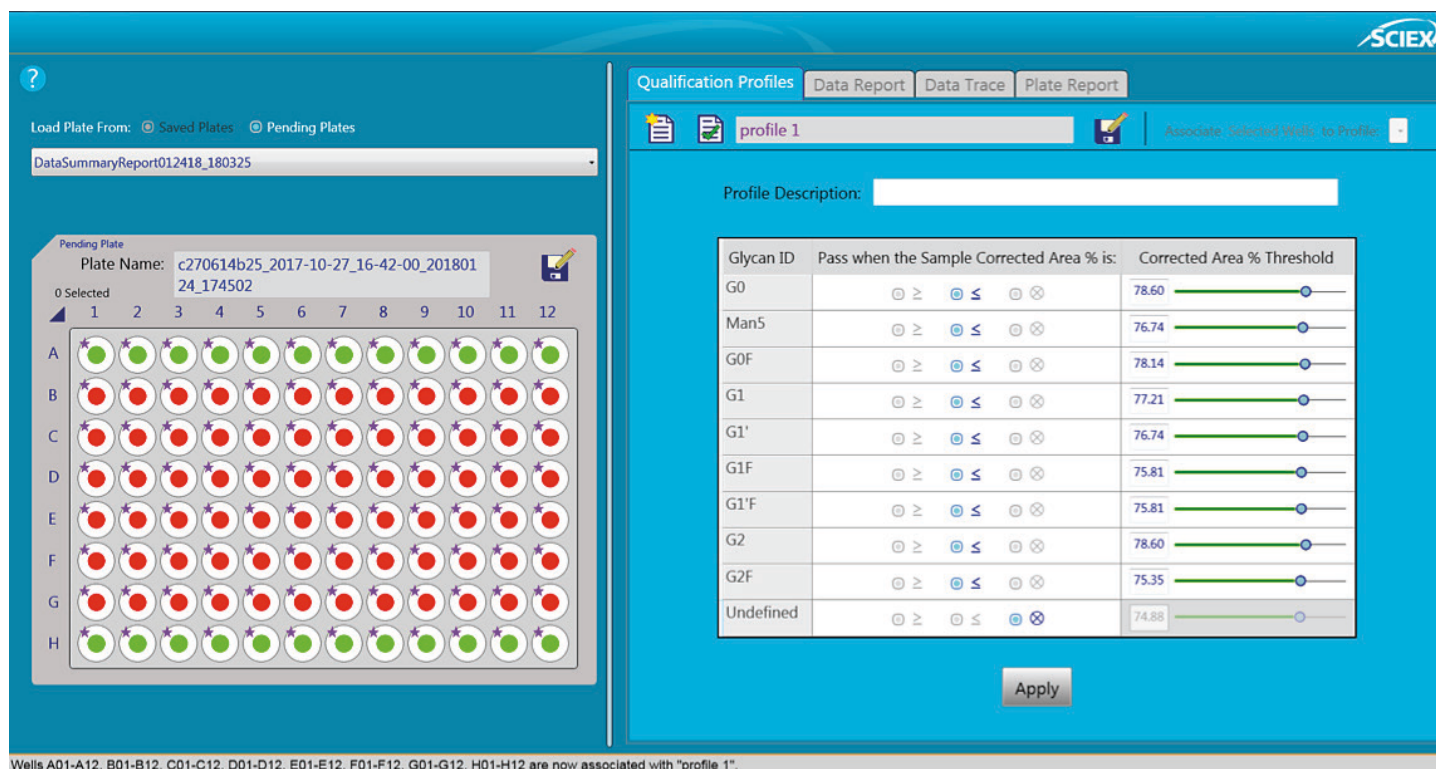


Figure 6. Qualification profile and dynamic pass/fail display of the wells on 96 well plate. Rows A and H – 9 glycan panel, rows B, D, and F – MAK33-Commercial purified antibody, and rows C, E, and G – IgG 1 k from Cell Culture Sample (protein A captured).

The second tab allows the user to access a data report. By simply selecting one well or multiple wells in the 96 well plate graphic, the Data Report tab will automatically update those with selected data. The third tab enables operators to visualize the data integration. The fourth tab provides access to a plate report which shows a summary of the plate with pass/fail criteria, the profile used to analyze the data, as well as the information in the data report.

Reproducibility

Figure 7 shows 12 separations of (A) the 9 glycan panel and (B) the N-linked glycans released from MAK33. Each graph represents one capillary.

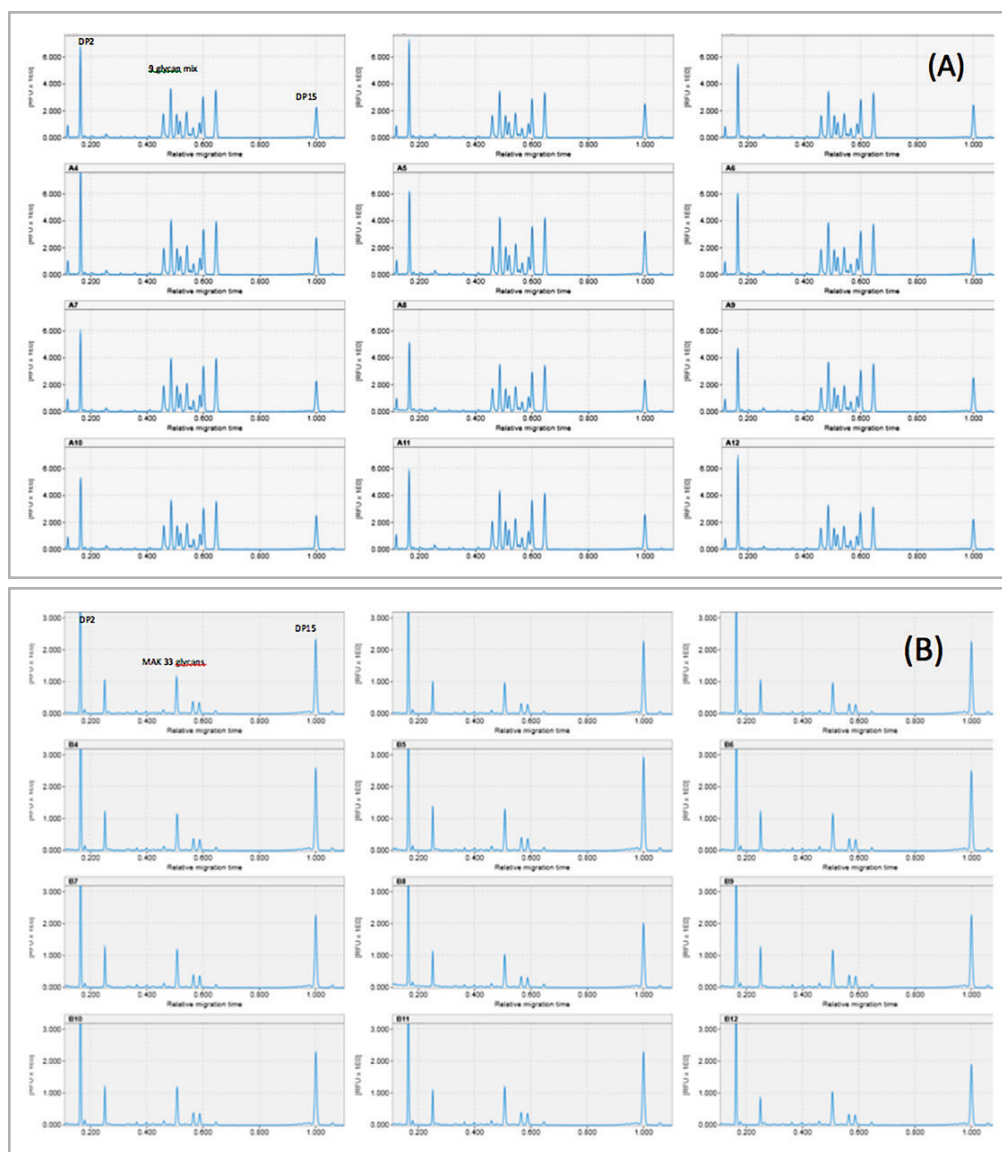


Figure 7. Reproducibility of runs: (A) 9 glycan panel and (B) MAK33 glycans.

Table 1 summarizes the reproducibility of glycan identification, corrected area % (CA%), and relative migration time (RMT) obtained for the 4 MAK33 glycans found. Glycans were identified with a RSD of 0.06% or better, with RMT RSD as low as 0.04% and CA% RSD as low as 0.25%.

Attribute	G0F	G1F	G1'F	G2F
Glycan ID (%RSD)	0.06	0.03	0.04	0.04
CA% (%RSD)	0.25	0.42	0.56	1.20
RMT (%RSD)	0.08	0.07	0.1	0.04

Table 1. Reproducibility of glycan identification, corrected area, and RMT for MAK33 glycans (n=36).

Drug Discovery and Development



Conclusion

Biopharmaceutical process development commonly requires analysis of hundreds to thousands of samples during clone selection and cell culture optimization. Lengthy analysis times associated with traditional glycan analysis techniques can create process bottlenecks and prevent timely in-process adjustments. The C100HT Biologics Analyzer uses 12 channel capillary electrophoresis separation with LED-induced fluorescence detection to quickly and reproducibly analyze N-glycans released from monoclonal antibodies (mAbs) and similar protein biologics. Its simple sample preparation and fully automated glycan identification capabilities facilitate the screening of large numbers of candidates quickly enough for in-process adjustments. The C100HT analyzer can process as many as five 96-well plates per day when automated liquid handling is incorporated into the workflow.

Who is SCIEX? SCIEX company's global leadership and world-class service and support in the capillary electrophoresis and liquid chromatography-mass spectrometry industry have made it a trusted partner to thousands of the scientists and lab analysts worldwide who are focused on basic research, drug discovery and development, food and environmental testing, forensics and clinical research.

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Publication number: RUO-MKT-02-7361-A 04/2018



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