LabChip GXII for High Throughput Characterization of Monoclonal Antibody Quality

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Abstract

Current initiatives in process analytical technology and quality by design are driving the need to identify and thoroughly understand the relationship between critical process parameters and product quality. Design of Experiment (DOE) studies can reveal the effect of cell culture changes on homogeneity, purity, and post-translational modifications of monoclonal antibodies but produce large number of samples. This trend can exceed the capacity of modern analytical technologies which primarily depend on HPLC and CE based separations to monitor protein and bio-therapeutic product quality. This increased number of samples requires a higher throughput analytical platform with automation and high precision. High throughput analyses of monoclonal antibodies have been performed on the Caliper’s LabChip GXII platform. Monoclonal antibodies were prepared under both reduced and non-reduced conditions. A Protein Chip and Microchip-CE-SDS was performed on Caliper's LabChip GXII System to identify and thoroughly understand the relation between critical process parameters and product quality. Design of Experiment (DOE) studies can reveal the effect of cell culture changes on homogeneity, purity, and post-translational modifications of monoclonal antibodies but produce large number of samples. This trend can exceed the capacity of modern analytical technologies which primarily depend on HPLC and CE based separations to monitor protein and bio-therapeutic product quality.

Materials and Methods

Reagents. Reagent kit was provided by Caliper Life Sciences. Dithiothreitol (DTT) and iodoacetamide (IAM) were purchased from Sigma-Aldrich.

Instrument and Microchip. Microchip-CE-SDS was performed on Caliper's LabChip GXII using Protein Express LabChip Kit. The chip was automatically printed on the instrument with polymer solution containing 0.3% SDS and fluorescent staining dye. The detection channels were filled with polymer solution line of SDS and dye.

Sample Preparation. 5 μL of each sample was combined with 35 μL sample buffer containing 0.7% LDS 1.9 mM Tris-Cl, 5.7% glycerol, and 1.7 mM EDTA. For reduced samples 24.5 μL of 1M DTT was added to 700 μL of sample buffer. For non-reduced samples 30 mM IAM was added to the sample buffer. Samples were heat denatured at 70 °C for 10 minutes. Afterwards, 70 μL of Milli-Q water was added to each sample well.

Assay Precision and Quantification

• Three Independent Operators
• Three Protein Chips
• Three LabChip GXII Instruments
• Three LabChip GXII Protein Chips

Determined by calculating the RSD of the percent purity of the intact mAb, 1,2. The integrated CE-SDS microchip based assay was used to separate proteins, determine sizes and quantify these monoclonal antibodies. Demonstration of the sensitivity, resolution, quantification, linearity, accuracy, and reproducibility of the platform is described.

Low Level Impurity Detection

• Ability to detect 1% impurity
• NANT at ≤1 μg/L under reducing and non-reducing conditions

- Lysozyme spiked at 1% of total protein concentration

Assay Precision:

- Determined by calculating the RSD of the percent purity of the intact mAb, 1,2.

Assay Quantification:

- Each mAb diluted to expected concentration of 1 μg/L.
- Quantification from a standard curve of normalized corrected area versus expected concentration of a rival.

Results are highly reproducible.

Conclusions

- LabChip-SDS on the LC/MS platform is a valuable tool for characterization of monoclonal antibodies and other proteins.
- Assay precision of intact mAb <0.5%.
- Good linearity was achieved: R2 = 0.9997 over the concentration range 7.8 μg/mL – 2 mg/mL.
- Ability to quantify low level impurities, to 0.040% range demonstrated.
- LabChip-GIX provides key advantages over conventional CE-SDS for screening and characterization of monoclonal antibody purity.
- High throughput automation platform
- Rapid flow-to-result
- 70 times faster than conventional CE: Fast separation time – 40 seconds per sample; 96-well plate in 1.25h
- Up to 400 samples per chip with single chip preparation
- Minimal sample volume, 2 μL
- Can assay on both crude and purified samples
- Minimal sample preparation required
- Capable of interfacing with plate robotics
- Can assay directly from a 96- or 384-well microtiter plate
- Automatic sampling directly from a 96- or 384-well microtiter plate

References

2. Data from Biotech client, used with permission.