Automation of Sample Preparation for CE-SDS-LIF of rMAbs with a Robotic Purification System

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INTRODUCTION

An increasingly used analytical method for protein biopharmaceuticals is capillary electrophoresis sodium dodecyl sulfate (CE-SDS) non-gel sieving analysis. However, CE-SDS with UV detection only provides the sensitivity of coomassie stained gels. To get the sensitivity of silver-stained gels the use of laser induced fluorescence is required. This requires the use of a manual and time-consuming dye-labeling protocol. The protein sample must be buffer-exchanged to provide the optimal conditions for fluorescence dye labeling followed by excess dye removal purification. A way to overcome this bottleneck is a method developed for a liquid handling robot. This allows the automation of the dye labeling protocol and SDS incubation as well as decreasing the amount of protein sample required by ten fold. This greatly increases throughput by reducing user input and decreases the amount of sample used. This approach is applied to rMAbs and comparability to previous methods will be demonstrated.

CURRENT METHODOLOGY

Sample Preparation Scheme for CE-SDS Analysis with LIF Detection

Buffer Exchange

Antibody Labeling

PhyTip media

Incubation with SDS

Buffer Exchange

PhyTip media

Antibody Labeling

PhyTip media

Incubation with SDS

Antibody Labeling

PhyTip media

Antibody Labeling

PhyTip media

Incubation with SDS

RESULTS

Buffer Exchange Optimization

<table>
<thead>
<tr>
<th>Sample Volume</th>
<th>Protein Amount Loaded (µg)</th>
<th>Air-Chase</th>
<th>Elution Volume (µL)</th>
<th>Percent Recovery</th>
<th>RSD%</th>
</tr>
</thead>
<tbody>
<tr>
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<td>48</td>
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<td>48</td>
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<td>200</td>
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</tbody>
</table>

CONCLUSIONS

The automated sample method for CE-SDS-LIF of rMAbs shown here, provides reproducible results that are comparable to the manual NAP-5 method with 10 times less sample.

The automation and scalability of the Phynexus system will meet current and future analytical needs.

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REFERENCES

Salas-Solano, 0., et al. Anal Chem. 2006; 78, 6583-6594.