Trace metal variability has been observed in cell culture products. SAFC’s raw material characterization program has identified that variability is typically caused by raw materials used in the manufacturing of these products. Even differences seen at ppb concentration can still potentially impact a biological system. Using an intact mass glycosylation assay, dose response, and Design of Experiment screening with an industry relevant CHO cell line, SAFC has evaluated the impact of 15 trace metals on our biological systems critical cell quality attributes. Statistical and multivariate analysis has been used to confirm the significance of any observed impact. The analysis has identified Fe and Mn at high concentrations can reduce the percent G0F and increase the G1F and G2F glycoforms. The effect of other trace metals on glycosylation was determined to be insignificant using ANOVA analysis (p values >0.05).

Introduction

Understanding protein glycosylation is very important in the development of therapeutic proteins. Any changes that may occur to these proteins’ critical quality attributes can result in difference in the products efficacy and safety. It has been well studied by the industry that specific metals can alter glycosylation. SAFC’s studies are designed to determine if the very low concentrations contributed by these raw materials would effect protein glycosylation profiles.

Biological Method

Screen 1:

Fifteen metals were evaluated in a high throughput 96 deep well plate dose response cell culture assay (Table 1). Eight different concentrations were screened for each of these metals using a CHO Zn™ K1 GS knock out cell line. The 96 deep well plates were cultured at 37°C for fifteen metals were evaluated in a high throughput 96 deep well plate dose response cell culture assay (Table 1). Eight different concentrations were screened for each of these metals using a CHO Zn™ K1 GS knock out cell line. The 96 deep well plates were cultured at 37°C for seven days with samples pulled days 3, 5, and 7 to analyze cell growth. A bioreactor was used to culture the cells at 37°C in a TPP Bioreactor tube system, which allowed for greater culture volume. The increased culture volume allowed for the raw materials used in the finished product manufacturing. These raw materials typically contain trace metals ranging from ppb to ppm concentrations. SAFC’s studies are designed to determine if the very low concentrations contributed by these raw materials would effect protein glycosylation profiles.

Analytical Method

The proteins were first purified using protein A affinity chromatography. These purified proteins were then analyzed for intact mass glycosylation using a Waters Aquity UPLC with a Waters Aquity UPLC PDA SEC column and Xevo QTof mass spectrometer. Data was then processed using Biopharmalynx software to quantitate percent G0F, G1F, G2F, non-glycosylated, and Man5 glycoforms.

Results and Discussion

Screen 1:

Table 1 summarizes each metal’s effect on protein glycosylation. Six of the 15 metals tested had no effect on protein glycosylation. Seven metals had slight differences that were not considered significant. More evaluation was necessary for these metals to prove any possible differences. Iron and manganese had significant effect on G0F, G1F, and G2F glycoforms (Figure 1 and 2).

<table>
<thead>
<tr>
<th>Trace Metal</th>
<th>G0F</th>
<th>G1F</th>
<th>G2F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe, Mn</td>
<td>4.33E-03</td>
<td>3.80E-15</td>
<td>6.47E-18</td>
</tr>
</tbody>
</table>

Table 1. Summary of metal effect on glycosylation (Trace Metals are coded A-N). Significant effect was seen with Fe and Mn. The question mark indicates any difference seen was not significant, but more data was needed to confirm results.

References and Acknowledgments
