Introduction to Case study

Problem: Appearance of two peaks in the purified protein product (protein Y) in RP_HPLC assay (Figure 6) and ratio changes over time.
Objective: To obtain product consistency - Protein Y

Challenges with two peaks in RP_HPLC

- Different batches have different peak 1/peak 2 ratio
- This ratio changes over time
- Peak 2 gets converted to peak 1 over time
- It is hard to use RP_HPLC assay to quantify and measure purity
- However, it has no impact on biological activity
- Mixture of two proteins has no impact on immunogenicity

Effect of Oxidizing agents and antioxidants on the two peaks

- Peak 1 appears only after during last diafiltration step in the process
- SM1 = 2 column purified
- SM2 = 3 column purified

Figure 7: SDS-PAGE analysis of Peak 1 and Peak 2 fraction showed that they have similar molecular weight

Conclusions

- Peak 1 is more oxidized form of Peak 2
- Oxidation process is irreversible
- Peak 2 only can be generated by including oxidation inhibitors
- Peak 1 only can be generated by oxidizing agents
- Purified product made could be prepared consistently with a single peak (peak 1 or peak 2)

Acknowledgements: Technologists and Scientists from Research and Product Development Departments at sanofi pasteur

Table:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>%Peak1 vs. %Peak2</th>
<th>Biological Activity</th>
<th>Immunological Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None*</td>
<td>6 / 94</td>
<td>+++</td>
<td>Yes</td>
</tr>
<tr>
<td>None</td>
<td>73 / 19</td>
<td>+++</td>
<td>Yes</td>
</tr>
<tr>
<td>20 mM Methionine</td>
<td>4 / 96</td>
<td>****</td>
<td>Yes</td>
</tr>
<tr>
<td>12 mM Thiouosphate</td>
<td>13 / 87</td>
<td>****</td>
<td>Not done</td>
</tr>
<tr>
<td>10 mM Sodium Periodate</td>
<td>100 / 0</td>
<td>*</td>
<td>Yes</td>
</tr>
<tr>
<td>10 um Periodate</td>
<td>99 / 0</td>
<td>*</td>
<td>Yes</td>
</tr>
</tbody>
</table>