生物药相似性评估的常规工作流程

使用台式X500B QTOF质谱仪进行曲妥珠单抗分析

Sibylle Heidelberger¹ and Sean McCarthy²
¹ 71 Four Valley Dr, Concord, ON L4K 4V8, Canada
² 500 Old Connecticut Path, Framingham, MA, 01701, USA

引言

生物药的研发过程是十分复杂的，在产品开发的过程中，需要大量的表征工作来确保药物的安全性和有效性。虽然有许多评估相似性的方法，但使用LC-MS进行完整蛋白分析可提供产品的异质性和杂质方面的相关信息。这种快速分析的能力使LC-MS检测成为开展其他研究之前的关键分析步骤。

在这里，我们展示了一种使用X500B QTOF系统高可重现性和耐用的分析生物药完整蛋白的方法，并使用BiopharmaView™软件进行简单快速的批处理分析。

实验条件和方法

生物类似药曲妥珠单抗由两处不同的制造商获得（标记，trast-1和trast-2）。样品使用0.2%甲酸稀释，或使用PNGase F（新英格兰生物实验室（伊普斯维奇，马萨诸塞州，美国））按标准流程去糖基化。

色谱条件

液相色谱系统为ExionLC™，蛋白质进样量为0.5 μg，使用Waters Acquity UPLC® Protein BEH C4柱，300A 1.7 µm、2.1 mm × 50 mm柱在80℃条件下分离。使用标准流动相（流动相A：水中0.1%甲酸，流动相B：乙腈中0.1%甲酸），色谱流速0.2-0.5 mL/min，总运行时间为5分钟。每次进样后前0.5 min切换至废液。

质谱条件

使用配备了Turbo V™离子源的X500B QTOF进行数据采集，large protein模式，扫描范围900-4000 m/z。电喷雾参数如下：

- Curtain gas: 35
- Ion source gas 1 (psi): 50
- Ion source gas 2 (psi): 50
- Temperature (°C): 400

结果和讨论

糖基化曲妥珠单抗

在这项研究中，我们使用了两种不同批次的曲妥珠单抗。我们从一个快速和简单的色谱方法开始，提供脱盐样品用于MS分析。使用X500B上的分流阀将色谱分离的初始部分排废液，脱盐后，切换阀门将样品注入MS源。如图1所示，色谱分离具有很高的重现性。

图1: 两个不同制造商的曲妥珠单抗高可重复性的色谱分离。

使用BiopharmaView™进行数据处理，采用曲妥珠单抗标准样品作为参照。
While evaluation of raw spectra is important to ensure that each replicate injection for each lot overlays very well. The replicate injections for each lot were plotted in a bar chart to display the relative abundances of each major glycoform, as shown in Figure 6. The plot shown was generated by comparing the second lot of trastuzumab against our initial characterized sample. Consistent agreement in the masses of each glycoform, however the intensity of post translational modifications rapidly. Each injection is in a different colour (blue, pink, red) and reflects the Gaussian distribution in m/z.

A batch analysis was submitted to compare the second lot of trastuzumab with the raw data. Our reconstructed spectra showed excellent agreement in the masses of each glycoform, however the intensity of post translational modifications rapidly. Matching the first antibody to an identified sample was tested (Figure 4). As shown there are some differences in the intensities of the glycoforms,

1. The reconstructed mass for trastuzumab in BioPharmaView. The first range of masses was selected which spanned the expected glycoform pattern.

2. Three replicate injections of trastuzumab were compared to a second lot of trastuzumab. The replicate spectra overlay very well. The replicate injections for each lot were plotted in a bar chart to display the relative abundances of each major glycoform. Figure 5: Comparison of glycoforms and intensities of the two lots. Lot 1 (1:Trastuzumab – 3:Trastuzumab) and lot 2 (4:Trastuzumab – 6:Trastuzumab).

3. A mirror plot image of one lot of trastuzumab (blue) vs a second lot of trastuzumab (pink) showing a distinct shift in the glycoform pattern.

4. The reconstructed mass for trastuzumab in BioPharmaView. The first range of masses was selected which spanned the expected glycoform pattern.

5. Two replicate injections of trastuzumab were compared to a second lot of trastuzumab. The replicate spectra overlay very well. The replicate injections for each lot were plotted in a bar chart to display the relative abundances of each major glycoform. Figure 5: Comparison of glycoforms and intensities of the two lots. Lot 1 (1:Trastuzumab – 3:Trastuzumab) and lot 2 (4:Trastuzumab – 6:Trastuzumab). Each injection is in a different colour (blue, pink, red) and reflects the Gaussian distribution in m/z.

6. Two batches of trastuzumab were compared to a second lot of trastuzumab. The replicate spectra overlay very well. The replicate injections for each lot were plotted in a bar chart to display the relative abundances of each major glycoform. Figure 5: Comparison of glycoforms and intensities of the two lots. Lot 1 (1:Trastuzumab – 3:Trastuzumab) and lot 2 (4:Trastuzumab – 6:Trastuzumab). Each injection is in a different colour (blue, pink, red) and reflects the Gaussian distribution in m/z.
回顾图3的结果，显示了主要糖型的强度变化，以及mannose-5（MAN5）种类存在的证据。绘制了该糖型与G1F图的对比图，以探究MAN5种类的水平。如图所示，与第二个样品相比，第一个样品中的MAN5峰更大。且重复分析结果一致。

图7. Man5与G1F丰度比对，批次1（1:Trastuzumab–3:Trastuzumab），批次2（4:Trastuzumab–6:Trastuzumab）。

在生物药生产过程中，批次间的比对是非常重要的工作。能够快速的对批次内或批次间样品进行比较可以有效监控和保证产品质量。台式的X500B QTOF质谱仪结合BioPharmaView™软件，非常适用于此类批次比对工作。本文中，BioPharmaView™软件通过样品独特的糖型特征，轻松快速地识别两个曲妥珠单抗生产批次之间的差异。该可视化工具可以使用户能够识别、量化和跟踪这些在生产批次之间的差异。