Automated Drug-to-Antibody (DAR) Calculations for Greater Efficiency in Analyzing Antibody Drug Conjugates (ADCs)

Biologics Done Right from Development to Production

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Key Benefits

- Software and hardware tools to facilitate the analysis and review of complex, high molecular weight species such as ADCs have been developed by SCIEX
- Large molecules such as monoclonal antibodies (mAbs) and ADCs can be separated from contaminants such as free drug, linker, and peptides/small protein fragments by Differential Ion Mobility with SelexION™.
- Data from species that have been selectively passed through SelexION™ can be reviewed easily within BioPharmaView™ Software. Each separate compensation voltage (CoV) is represented as a discrete experiment.
- The routine use of differential ion mobility is accessible to scientists at all stages of biopharmaceutical development to help tease out differences that would otherwise remain obscured by matrix or other contaminants.

Introduction

In 2013 the US FDA approved the Antibody Drug Conjugate (ADC) Kadcyla as a new therapy for patients with late stage metastatic breast cancer [1]. This was not the first such molecule in its class to be approved, but such is the importance of these biotherapeutics that it was reviewed under the FDA's priority review program [1]. Trend analysis of novel new drug approvals by the FDA showed that in 2014 41 novel drugs were approved. This was more than the average annual approvals over the last decade [2]. As this trend continues, and the complexity of biopharmaceutical molecules continues to increase, developers of ADCs need to find better ways of coping with their complex datasets. This Technical Brief outlines a new tool from SCIEX that facilitates the rapid assessment of DAR for ADCs.

Results

Using TripleTOF® technology, separations systems such as CESI, and MicroLC, and data processing with BioPharmaView™ software, an analyst can provide a DAR readout within seconds. Calculations using peak areas that may have previously taken hours to complete are now ready to report instantly. The readouts can be obtained by an analyst with no expert training. Therefore BioPharmaView™ software facilitates method development in less time, and provides higher sample throughput. Faster review helps an organization develop a robust and reproducible

Figure 1: An automated DAR Calculation for a Biotherapeutic in Development. The screenshot shows a tabular readout of the DAR (left), a bar graph in the centre, and a chromatographic readout of the identified species on the right.

DAR method [4]. Although peak detection is rapid, exporting the data to separate spreadsheets and setting up calculation mechanisms is time consuming and adds multiple steps that increase risk of human error. For example an analysis where there are four chromatographic peaks, each requiring 15 minutes of analysis for DAR, and 10 samples to analyze, would require about 10 hours. Using new functionality in BioPharmaView™ software reduces this analysis time to approximately 10 minutes, including reporting the data out to a document ready for review.

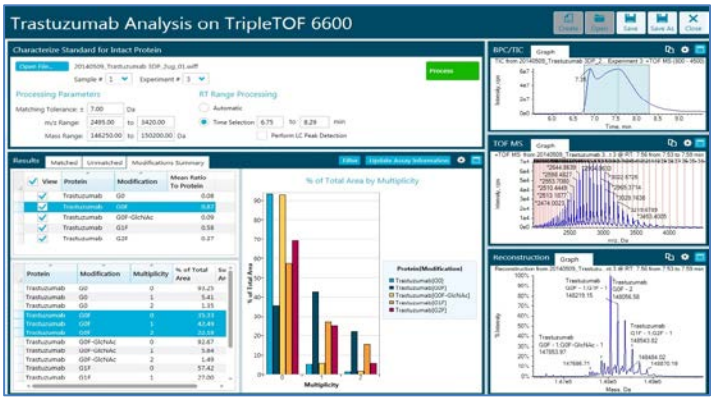

Figure 2: Automated Functionality to show relative proportions of modifications to intact Humira.

Figure 1 shows the ESI-MS spectrum of a trial intact lysine-linked ADC based on an IgG molecule. The screenshot shows how an analyst can simply read out a DAR calculation from an intact molecule, which is ready to send to a report immediately. In the top left hand panel is a tabular readout of the DAR with the assigned number of attached drugs clearly shown and a 'mean ratio' calculated. The corresponding visual display is in the central panel, with a bar graph denoting how many drugs are attached. In this example, the distribution is between 3 and 10. In the three right hand panels are the chromatographic, raw, and deconvoluted data respectively, from top to bottom. In the raw data in the top panel the blue area indicates which (chromatographic) peak area has been used to make the calculations. Using a mouse 'drag' the user can simply select different chromatographic regions to work out retention time variability of isoforms present in a sample. The graphs are updated automatically.

The same functionality can also be applied to other modifications, such as glycoforms or terminal modifications. In Figure 1, PyroGlutamic acid conversion is calculated as one of the assigned modifications. In Figure 2, a number of glycoforms are tracked simultaneously, showing all the modifications that are assigned automatically by BioPharmaView™ software. Note that the user may also filter the results according to their needs and display only those modifications which they need to report. A number of filter criteria are available to the user which may be used to provide reportable information on specific quality attributes.

Discussion

Early in development, analysts are tasked with providing rapid feedback to their synthetic chemists on how well a conjugation strategy may have worked. This allows the synthetic chemists to select optimal methods. The faster the feedback, the faster development can proceed. Researchers working in this field have identified DAR as "one of the most important quality attributes of an ADC" because this "determines the payload that can be delivered to the tumor cell and can directly affect both safety and efficacy" [3].

During later development and subsequent production, analysts need means to rapidly confirm that the product retains the expected characteristics. Therefore the ability to respond rapidly with an accurate readout of DAR allows potential problems to be identified early and allows development to become more efficient. If DAR calculations are used as part of release assays, rapid and robust measurements can help an organization maintain the integrity of a product. The task can be complicated by the heterogeneity of the constructs, the number of impurities (or fragments if the synthesis is still at an early stage of optimization), difficulty in

chromatographic separations, or interfering compounds such as those found in formulation.

Conclusions

SCIEX has continued to innovate to allow analysts to quickly and accurately perform their scientific work, and save hours of time. For a representative sample set, the savings amount to an entire working day, allowing a scientist more time to devote to more productive scientific questions. The rapid, simple application of the automated tool in BioPharmaView™ software facilitates the analysis of Intact ADCs to allow researchers to:

- Immediately determine the intact molecular weight of the construct
- Obtain Drug-Antibody Ratio (DAR)
- Adjust processing parameters instantly
- Determine the range of the number of drugs linked
- Provide a general purpose tool that provides information on modifications of intact species.

For complex biologics species such as ADCs, SCIEX provides a toolkit that provides analysts with instant, automated readouts of quality attributes, irrespective of the mode of separation, and the presence of an ion mobility device.

References

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