# Enhanced Analysis of Antibody Drug Conjugate (ADC) Candidates in Development Using Ion Mobility Separation

***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

ADCs are one of the fastest growing segments of the biotherapeutic pipeline, and a number of candidates are in development. Many ADCs currently under development are for oncological indications, relying on the monoclonal antibodies targeting various types of cancer [1, 2]. ADCs present challenges for analytical scientists because of their inherent heterogeneity and high molecular weight, as well as the fact that they are exposed to many more reagents (contaminants) than a typical mAb preparation. ADC development is more than just a simple analysis of its parts, with a history of challenging analytical conditions [3]. Therefore developers need to closely monitor fine details of the ADCs that they are synthesizing to better understand these complex therapeutics. Adding an additional dimension of ion mobility separation helps differentiate between species that are not easily separable by mass spectrometry-friendly LC methods.

In this Technical Brief we illustrate how TripleTOF® technology coupled with SelexION™, SCIEX MicroLC systems, and BioPharmaView™ software can determine different DAR values automatically from species separated simultaneously by liquid chromatography (LC) and differential mobility (DMS). Without SelexION™ it would be impossible to distinguish between the distributions of drugs linked to the antibody in different forms that co-elute chromatographically. BioPharmaView™ software has been adapted to allow deconvolution and automated display of Drug to Antibody Ratio (DAR) for each CoV channel. Assays that may have needed days to complete are now ready to report within minutes, with a greater depth of information and no penalty to the LCMS method used.


**Figure 1: BioPharmaView(TM) software screenshot showing the panels relevant to the analysis. From left top clockwise: processing parameters, chromatogram, raw data, deconvoluted data, tabular summary.**

For an analyst it is critical to understand the heterogeneity of their constructs, and the number of impurities or fragments if the synthesis is still at an early stage of optimization. They also need to cope with interfering compounds such as those found in formulation.
Assays at the intact molecule level for ADCs are well-established as important measures of the quality attributes of the potential therapeutic product. Typical assays determine the intact molecular weight of the construct, obtain the Drug-Antibody Ratio (DAR), and to determine the range of the number of drugs linked [4]. Analysts also need to know very clearly how robust an assay might be if they plan to transfer it to QA/ QC. Therefore obtaining a complete understanding of the distribution can be extremely important. Even with judicious sample preparation, and fraction collection, the overall distribution, and the species synthesized, might be tedious to determine. Therefore use of SelexION™ offers an alternative method that can be as informative as other techniques in a far shorter time period.

## Results

Figure 1 shows the ESI-MS spectrum of a non-commercialized intact ADC based on an IgG1 molecule. In the spectrum there are a number of interfering species, some of which are visible at the lower end of the m/z scale below approximately 2500 m/z. The deconvolution of this spectrum provided a DAR distribution with species assigned appropriately. Figure 2 shows how the DAR calculations can be obtained automatically for such an analysis where a straightforward LC method is applied.

However, the application of SelexION™ technology provided much needed clarity. SelexION™ is a simple, differential ion mobility device that fits at the source of the mass spectrometer. SelexION™ does not interfere with the analysis, and provides an orthogonal separation based on ion mobility. A number of attributes make this an ideal addition to the analysis:

- Simple Voltage changes are applied instantly
- No user expertise is required, and no need for complex tuning
- Separation occurs PRIOR to detection, so no interference with data processing
- An identical (LC) method can be used for all cases

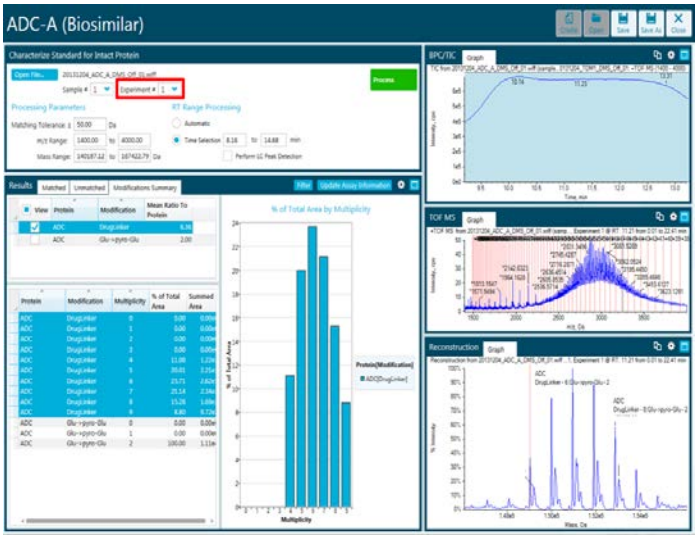


**Figure 2: Screenshot of ADC intact analysis as per Figure 1, but with the tab of 'Modifications Summary' displayed. The DAR calculations are made automatically and displayed both as a graph showing the distribution, as well as a table summarizing the DAR with %.**

Functionality added to BioPharmaView™ software allows the processing of channels associated with different CoV values. This is shown in Figure 3.
The Lysine-linked ADC studied here eluted from the DMS device at a CoV around -5V. Smaller contaminants and contaminating fragments elute from the DMS device approaching positive CoV values [data not shown]. Retention time and all other instrument parameters were held constant for all sample acquisitions.

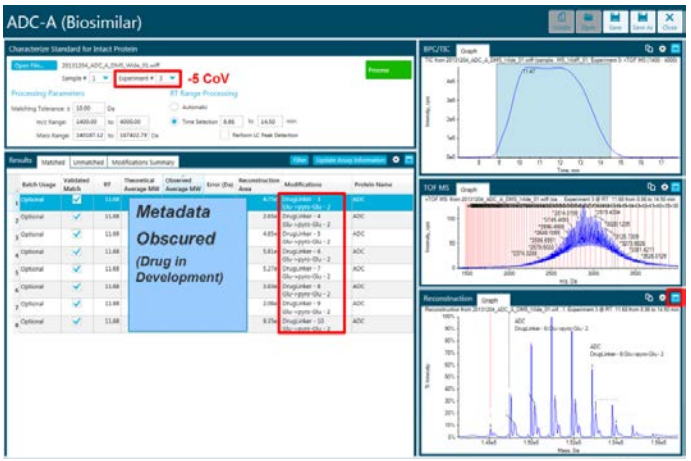


**Figure 3: Screenshot of a single channel analysis of an intact ADC with a selected CoV of differential Ion Mobility selected for the optimal value.**

Figure 4 is the same data as Figure 3, but showing the graphical distribution of the species available in that CoV channel. Comparing the mean ratios of the average of all species together (Figure 2 – where no

SelexION™ was applied) to those at the optimal CoV (figure 4 – CoV at -5V) shows that the values are different. In this case CoV at -5V revealed a species that was not detectable when no SelexION™ voltage was applied. The overall DAR values are slightly different, and the actual peak areas of the species are available to the analyst to determine where the reasons for the discrepancies. In this case it is clear that interfering species had made the determination more challenging.

## Conclusions

Without any additional sample preparation, or specialist knowledge of the molecule, we were able to show that the application of SelexION™ revealed previously unknown information about a complex ADC in development.

For complex biologics species, SelexION™ on a TripleTOF® instrument from SCIEX may provide a simple, elegant solution to cleaning up intact species from an ESI spectrum.

The rapid, simple application of Ion Mobility facilitates the analysis of Intact ADCs and

- Has no effect on other experimental conditions
- Uses identical processing parameters/ informatics
- Is applicable with no expert training
- Is transferable across ion-mobility platforms with SelexION™ such as QTRAP® technology.


Figure 4: DAR table and distribution for an ADC molecule in development showing the utility of SelexION™ to revealing previously unseen species.

## References


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