HIGH SENSITIVITY INTACT ANTIBODY DRUG CONJUGATE ANALYSIS USING AN INTEGRATED MICROFLUIDIC DEVICE COUPLED TO HIGH RESOLUTION MASS SPECTROMETRY

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OBJECTIVE

To characterize the analytical capabilities of the ionKey/MS system for the mass determination of antibodies and antibody drug conjugates.

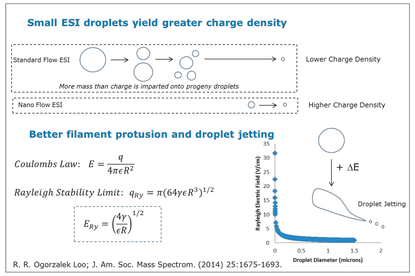
INTRODUCTION

The iKey separation device is packed and assembled using reversed-phase bridged ethyl hybrid (BEH) 1.7µm C4 particles. The iKeys are integrated with an ESI emitter shown in Figure 1 below; iKey dimensions were 150 µm id x 10 cm. In addition to the iKey, trapping columns, 300 µ m x 5 cm, were packed with either BEH 5.0 µ m C4 particles or MassPrep desalting column technology. These columns were plumbed to the trapping valve manager (TVM), which was consequentially plumbed to the iKey.

These dimensions provide improved sensitivity compared to identical injection volumes on an ACQUITY UPLC 2.1 mm column. Much of this sensitivity improvement is based on improvements in a) ionization efficiency, b) sampling efficiency, and c) ion suppression.

Electrospray ionization efficiency is inversely related to flow rate. Generally, as flow rates are reduced, electrospray efficiency increases non-linearly. The major difference between microflow ESI and high flow ESI is the diameter of droplets that are generated between the two flow regimes. In the microflow regime, it is possible to generate smaller droplets as compared to the high flow. This assists in electrospray ionization by enabling a relatively high charge density within a droplet, and also increasing the effective electric field at the surface of the droplet. Since there is a proportionally higher analyte to solvent ratio in the spray plume for microflow ESI, the sampling of a greater fraction of the analyte will occur. Finally, ion suppression is reduced in smaller droplets because the ions have an "easier" path to the surface of the droplet.

As is the case with monoclonal antibodies, improvements in electrospray efficiency will further assist in the reduction of the barrier of entry into the gaseous phase for large macromolecules.



R. R. Ogorzalek Loo; J. Am. Soc. Mass Spectrom. (2014) 25:1675-1693.

Figure 1. Illustrates the advantages of small droplet ESI for charge density (top) and filament protusion and droplet jetting (bottom).

METHODS

Sample Preparation

Samples were dissolved in 3% acetonitrile with 0.1% formic acid. A variety of sample prep methodologies were tested in combination with the instrument described here.

Trapping Conditions with ionKey/MS

TVM:	ACQUITY M-Class TVM
Trap Column:	300 µm x 100 mm C4 @ 80 °C
Trap Time:	2 min
Trap Flow Rate:	15 µL/min
Loop Volume:	5 µL
Isocratic Cond:	3% MPB, 97% MPA
Mobile Phase A:	Water + 0.1% Formic Acid
Mobile Phase B:	Acetonitrile + 0.1% Formic Acid

TVM:	ACQUITY M-Class TVM
Trap Column:	300 µm x 100 mm Desalting Chemistry @ 25 °C
Trap Time:	2 min
Trap Flow Rate:	15 µL/min
Loop Volume:	5 µL
Isocratic Cond:	3% MPB, 97% MPA
Mobile Phase A:	Water + 0.1% Formic Acid
Mobile Phase B:	Acetonitrile + 0.1% Formic Acid

Gradient Conditions with ionKey/MS

BSM:	ACQUITY M-Class BSM
iKey:	150 µm x 100 mm C4 @ 80 °C
Trap Flow Rate:	3 µL/min
Loop Volume:	5 µL
Gradient:	3% MPB to 95% MPB in 3.5 min
Mobile Phase A:	Water + 0.1% Formic Acid
Mobile Phase B:	Acetonitrile + 0.1% Formic Acid

MS Conditions with ionKey

MS System:	Xevo G2-XS QTOF
Ionization Mode:	ESI positive
Capillary Voltage:	3.5 kV
Sampling Cone:	150 V
Source Temp:	150 °C
Desolvation:	None applied
Mass Range:	*m/z* 500 – 4000

Figure 2. (top) Series of ionKey devices illustrating integrated chromatography-ESI. (bottom) Plug and play ionKey source illustrating insertion of iKey devices.

RESULTS

The charge state distribution has different characteristics depending on the ESI spray conditions. Figure 3 illustrates the difference between a high flow charge state distribution and a low flow charge state distribution. Generally, as the flow rate is decreased droplet diameter will decrease and become more monodisperse. Since droplet size has been implicated in charge state the monodisperse nature of the spray will affect the charge state distribution of the antibody or antibody-drug-conjugate.

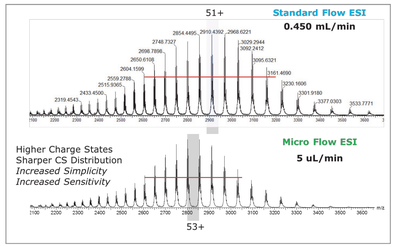


Figure 3. (top) Standard Flow ESI of mAb. (bottom) Micro Flow ESI of mAb.

The chromatographic performance of the 2D trapping methodology relied on the differential refocusing between the trap and the analytical iKey. Our best performance was established between a trap packed with the MassPrep desalting sorbent and a C4 ionKey that was packed with bridged ethyl hybrid C4. Since the desalting sorbent is less retentive than the C4 it was possible to refocus onto the analytical ionKey and generate a very sharp antibody peak measuring 2.58 seconds at 10% of the peak height. The characteristic chromatography is illustrated in Figure 4 below.

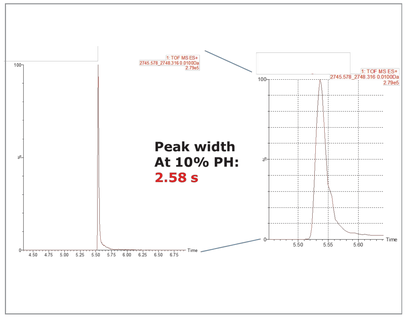


Figure 4. (left) Full view of the chromatographic separation from the XIC extraction. (right) zoomed view of the mAb peak illustrating peak width at 10% peak height.

Given the excellent chromatographic performance of the system it was possible to perform quantitative analysis across two orders of magnitude for glycosylated mAb, deglycosylated mAb, glycosylated ADC and deglycosylated ADC. Figure 5 illustrates the chromatogram and m/z charge state distribution for a fully deglycosylated mAb.

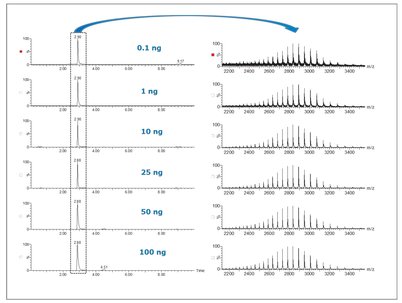


Figure 5. (left) Chromatogram of a deglycosylated mAb for mass loads of 0.1 ng to 100 ng (on-column). (right) Charge state distribution of the Waters mAb across mass load.

Lower limits of detection for the mAb and ADC were in the range of 0.1 to 1 ng (on-column). Variables such as glycan heterogeneity and sample composition vary the limits of detection dramatically. Mass accuracy was unaffected from the lower limit of detection to the high load limits of the device. Figure 6 illustrates the comparison between 50 ng and 1 ng for the fully glycosylated mAb standard, and the differences in mass accuracy between these two different mass loads.

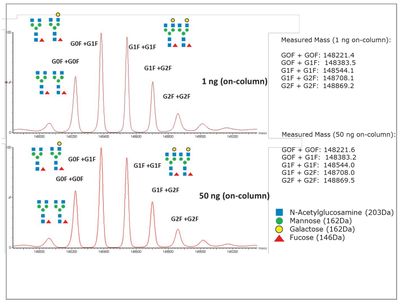


Figure 6. Deconvoluted spectra for two mass loads on column (1ng and 50 ng).

Robustness and reproducibility are also important variables in mAb and ADC determination. We tested a series of mAb standard injections onto an ionKey tile for reproducibility and found the peak retention reproducibility to be less than 0.1% RSD, while peak area reproducibility across this replicate was less than 10% RSD. Figure 7 illustrates both peak area and retention time reproducibility across the injection series.

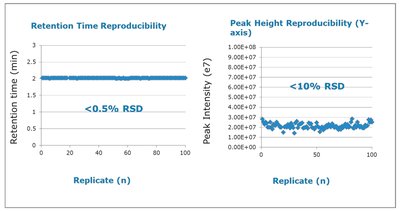


Figure 7. Retention time and peak height reproducibility for the injection of mAb standard

The high throughput capabilities of this instrument enable sub 5 min cycle time analysis of mAb, specifically for glycan analysis. Figure 8, illustrates the high throughput capabilities of separating a mAb and performing glycan analysis. It is important to note that when mAb and ADC's are not correctly desalted, the result is a charge state distribution that is indecipherable with respect to the glycan analysis. Although the separations are performed rapidly in Figure 8, the glycan structure is present, which suggests adequate desalting and separation.

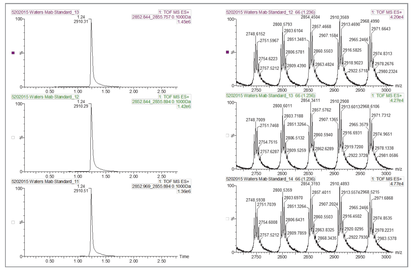


Figure 8. Retention time and peak height reproducibility for the injection of mAb standard

In addition to mAb analysis we also investigated analysis using this instrument for ADC. Specifically, we investigated trastuzumab for linear range, detection limit and mass accuracy. Figure 9 illustrates the comparison of the spectral quality for the fully glycosylated ADC over a series of mass loads onto the ionKey system. Accurate mass was identified for both the trastuzumab, linker and drug that was within 0.5 Da from the literature values. Furthermore, using the high sensitivity capabilities of the ionKey system it was possible to identify linkers that had released payload, yet not be cleaved. Linearity was determined to be 3 orders of magnitude for this ADC, but was variable depending on the ADC and the matrix components.

In addition to Trastuzumab we have also investigated a wide variety of other proprietary ADC and glycosylated mAb compounds including lysine conjugated ADCs and Humira. These compounds have also been purified from complex matrix, including rat plasma, using generic affinity capture methodologies.

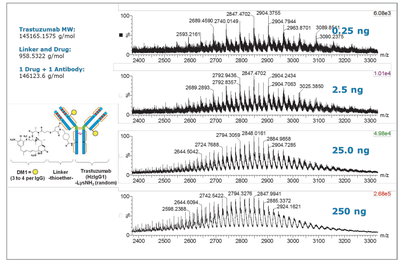


Figure 9. Trastuzumab separated and deconvoluted over a series of mass loads, accurate mass of parent mAb, linker and drug was identified.

CONCLUSION

- **Robust and reproducible method with limited sample preparation required for sensitive analysis of mAb and ADC.**
- **LLOD ranging from 0.1 to 1 ng (on-column) and demonstrated a 10X improvement in sensitivity over standard flow methods.**
- **Trapping enabled improved mass load capabilities and refocusing improved peak shape.**
- **The advantages of the ionKey/MS system for mAb and ADC analysis include: improved sensitivity, reduced sample consumption, ease of integration for high throughput analysis, ease of use, and reduced solvent consumption and operating costs.**

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