Applying Multi-Attribute Method for IEX fractionated DART® molecule for Stability Assessment

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Introduction

- DART molecules are novel therapeutics designed to enable the cancer fighting properties of immune effector cells
- The DART molecule characterized here is a heterodimeric protein composed of two chains: (Chain 1/ E-coil and Chain 2/ K-coil) (Figure 1)
- The inhouse LC-MS method deployed for characterization of the DART molecule used IEX fractionated protein digested using trypsin
- The introduction of MAM following IEX enables detection of impurity/ new peaks in the samples, the relative change of each impurity and their properties (acidic/basic nature)

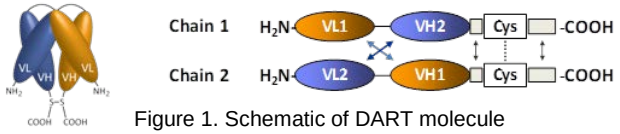
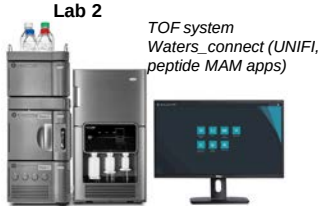


Figure 1. Schematic of DART molecule

Experimental

- A group of 7 IEX fractionated samples and source material were used in DART® molecule PTM/ impurity analysis
- Lab 2 performed a blind sample analysis for the IEX fractions
- Concentrations of each IEX fraction was adjusted to the lowest fraction prior to digestion. The samples were reduced, alkylated and tryptic digested using a *RapiGest*™ assisted digestion method
- Data was acquired in Lab 2 (@ Waters Corporation, Milford) using a RPLC-TOF-MS system
- The peptide attributes were identified using waters_connect™ software
- %Modification levels and new peaks were generated using the Peptide MAM App

Lab 2



TOF system Waters_connect (UNIFI, peptide MAM apps)

Results

Workflow

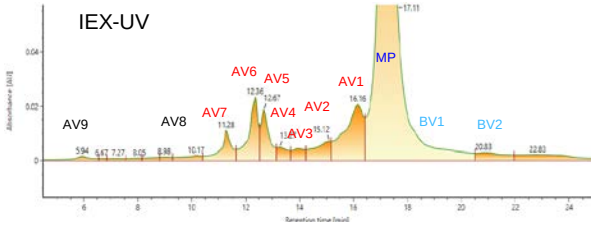


Figure 2. The source material sample was fractionated by IEX-UV. Fractions AV5-BV1 were analyzed by LC-MS for PTM identification

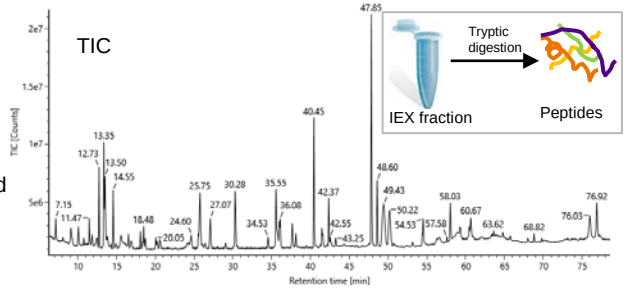


Figure 3. The IEX fractions were digested with trypsin using a *RapiGest assisted digestion method*. Samples were analyzed by RPLC-MS[E]

Attribute monitoring

Table 1. The %modification levels calculated by Peptide MAM App for combined N31 deamidation (CDR1 VL) level of chain 2:T3 peptide

Sample	Lab 2
Source material	2.3 %
AV5	4.2 %
AV4	4.2 %
AV3	1.3 %
AV2	13.6 %
AV1	0.1 %
MP	0.2 %
BV	1.3 %

Number of New Peaks Detected

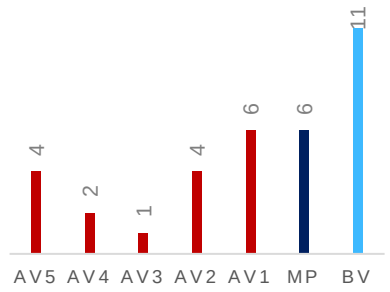


Figure 4. The new peaks detected by MAM software compared to source material (reference). The new peaks are flagged if there were >10 fold-change in MS response relative to the reference

Results

Inter-laboratory data comparison

Lab 1

QTOF system UNIFI™ software

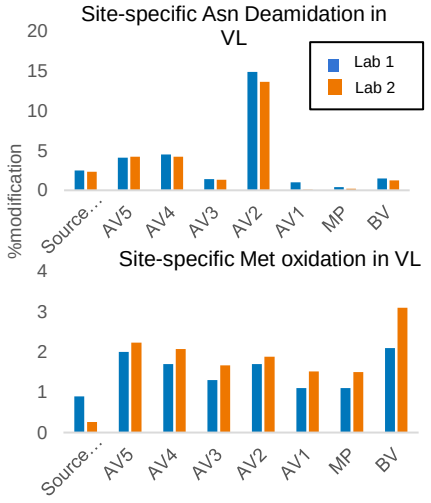


Figure 5. The %modification levels were calculated for two selected attributes of the DART® molecule using waters connect, Peptide MAM App. The attributes selected are in the Chain 2, VL region. The data was compared to previously generated data by Lab1 (different instrument, method and operators). Peptide mapping method and manual %PTM calculations were used by Lab 1 for the analysis

Conclusions

- The IEX fractionation of the DART® molecule generated 5 acidic variants (AV1-AV5), main peak (MP) and a basic variant (BV). Sample preparation was specifically designed for low concentration of protein fractions
- The optimized method was capable of analyzing these fractions by LC-MS (in DIA mode) using Peptide MAM App
- Lab 2 generated % PTM data using the Peptide MAM app. The results were highly comparable to that from Lab 1 generated by manual data analysis
- MAM App also found new peaks, suggesting the presence of unique components in each tryptic digested IEX fraction. The new peaks showed changes associated with untargeted peptide modifications compared to the reference sample. Validation of these new peaks are planed as the next step.

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