

CLAIMS

What is claimed:

1. A method of determining a ratio of drug to antibody (DAR) in a sample comprising an antibody-drug conjugate, comprising:

applying the sample to a size exclusion chromatography matrix;

detecting absorbance response of the sample at a first light wavelength (λ_1), wherein the first light wavelength is a predetermined absorbance maxima of the antibody;

detecting absorbance response of the sample at a second light wavelength (λ_2), wherein the second light wavelength is a predetermined absorbance maxima of the drug;

determining a total absorbance of the sample at the first light wavelength and a total absorbance of the sample at the second light wavelength, each total absorbance calculated by integrating peaks of absorbance responses over a time interval; and

calculating the DAR using the following Equation 1:

$$\text{DAR} = \frac{\epsilon_{\lambda_2}^{\text{mAb}} * \text{Total Area}_{\lambda_1} - \epsilon_{\lambda_1}^{\text{mAb}} * \text{Total Area}_{\lambda_2}}{\epsilon_{\lambda_1}^{\text{drug}} * \text{Total Area}_{\lambda_2} - \epsilon_{\lambda_2}^{\text{drug}} * \text{Total Area}_{\lambda_1}}$$

wherein $\epsilon_{\lambda_1}^{\text{mAb}}$ is an extinction coefficient of the antibody at the first light wavelength; $\epsilon_{\lambda_2}^{\text{mAb}}$ is an extinction coefficient of the antibody at the second light wavelength; $\epsilon_{\lambda_1}^{\text{drug}}$ is an extinction coefficient of the drug at the first light wavelength; $\epsilon_{\lambda_2}^{\text{drug}}$ is an extinction coefficient of the drug at the second light wavelength; Total Area $_{\lambda_1}$ is the total absorbance of the sample at the first light wavelength; and Total Area $_{\lambda_2}$ is the total absorbance of the sample at the second light wavelength.

2. The method of claim 1, further comprising calculating the extinction coefficient of the antibody at the first light wavelength or at the second light wavelength based on absorbance of a solution of the antibody with a known concentration.

3. The method of claim 1, further comprising calculating the extinction coefficient of the drug at the first light wavelength or at the second light wavelength based on absorbance of a solution of the drug with a known concentration.
4. The method of any one of claims 1-3, wherein the size exclusion chromatography comprises ultra-performance liquid chromatography (UPLC), a reversed phase (RP) UPLC, or a high performance liquid chromatography (HPLC).
5. The method of any one of claims 1-4, wherein the sample includes 1 $\mu\text{g}/\mu\text{L}$ to 500 $\mu\text{g}/\mu\text{L}$ of antibody-drug-conjugate (ADC).
6. The method of any one of claims 1-5, wherein the antibody is an anti-tumor antibody.
7. The method of any one of claims 1-6, wherein the drug is a pro-apoptotic, cytostatic or cytotoxic agent.
8. The method of any one of claims 1-7, wherein integrating peaks of absorbance responses includes detecting a peak shoulder of each peak of absorbance response.
9. The method of any one of claims 1-8, wherein integrating peaks of absorbance responses includes summing the area of high molecular weight (HMW) peaks, low molecular weight (LMW) peaks and a main peak.
10. The method of any one of claims 1-9, wherein the time interval is between 3 to 9 minutes.