

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 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 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 claim 1, 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 claim 1, 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 claim 1, wherein the antibody is an anti-tumor antibody.
7. The method of claim 1, wherein the drug is a pro-apoptotic, cytostatic or cytotoxic agent.
8. The method of claim 1, wherein integrating peaks of absorbance responses includes detecting a peak shoulder of each peak of absorbance response.
9. The method of claim 1, 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 claim 1, wherein the time interval is between 3 to 9 minutes.
11. A method of determining a ratio of drug to antibody (DAR) in a first sample comprising an antibody-drug conjugate, comprising:
 - measuring total absorbance of the first sample comprising the antibody-drug conjugate by:
 - applying the first sample comprising the antibody-drug conjugate to a size exclusion chromatography matrix;

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

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

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

measuring total absorbance of a second sample comprising the antibody by:

applying the second sample comprising the antibody to a size exclusion chromatography matrix;

detecting absorbance response of the second sample comprising the antibody at the first light wavelength (λ_1);

detecting absorbance of the second sample comprising the antibody at the second light wavelength (λ_2); and

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

calculating a DAR using the following Equation 2:

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

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

total absorbance of the second sample comprising the antibody at the second light wavelength; Total Area _{λ_1} ^{ADC} is the total absorbance of the first sample comprising the antibody-drug conjugate at the first light wavelength; and Total Area _{λ_2} ^{ADC} is the total absorbance of the first sample comprising the antibody-drug conjugate at the second light wavelength.

12. The method of claim 11, 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.

13. The method of claim 11, 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.

14. The method of claim 11, wherein the size exclusion chromatography comprises ultra-performance liquid chromatography (UPLC), a reversed phase (RP) UPLC, or high performance liquid chromatography (HPLC).

15. The method of claim 11, wherein the first sample includes 1 $\mu\text{g}/\mu\text{L}$ to 500 $\mu\text{g}/\mu\text{L}$ of antibody-drug-conjugate (ADC).

16. The method of claim 11, wherein the antibody is an anti-tumor antibody.

17. The method of claim 11, wherein the drug is a pro-apoptotic, cytostatic or cytotoxic agent.

18. The method of claim 11, wherein integrating peaks of absorbance responses includes detecting peak shoulder of each peak of absorbance response.

19. The method of claim 11, 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.

20. The method of claim 11, wherein the time interval is between 3 to 9 minutes.

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

placing the sample in a vessel;

moving a probe relative to the vessel such that the probe makes contact with the bottom of the vessel;

moving the probe relative to the vessel such that the probe moves from the bottom of the vessel through the sample by a predetermined increment such that a preselected path length through the solution is obtained;

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

repeating steps of moving the probe relative to the sample and taking a measurement at the first wavelength;

generating a regression line from the absorbance at the first wavelength and path length such that a slope of the regression line is obtained;

determining the concentration of the antibody by dividing the slope of the regression line by an extinction coefficient of the antibody at the first wavelength;

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

repeating steps of moving the probe relative to the sample and taking a measurement at the second wavelength;

generating a regression line from the absorbance at the second wavelength and path length such that a slope of the regression line is obtained;

determining the concentration of the drug by dividing the slope of the regression line by an extinction coefficient of the drug at the second wavelength; and

calculating a DAR using the determined concentration of drug and the determined concentration of antibody.