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Improving Detection Limits of ADC Free-Drug Impurities Using the ACQUITY QDa Mass Detector

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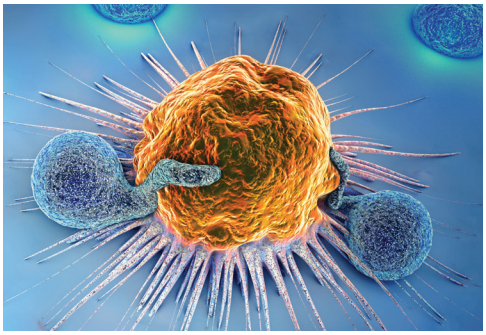

The ACQUITY QDa Detector offers increased sensitivity and dynamic range in the detection of trace free-drug impurities in formulated ADC samples.

GOAL

To demonstrate the Waters® ACQUITY® QDa® Detector can increase the dynamic range and detection limits of UV-based impurity assays for formulated ADC samples.

BACKGROUND

As a new class of immunoconjugate therapy, antibody-drug-conjugates (ADCs) have received increasing attention for their novel modality. Conceptually, the cytotoxic payload, which is covalently linked to the antibody, is delivered in a targeted manner to cancerous tumors via the specificity of the antibody. This unique modality increases the therapeutic window for highly potent anti-cancer drugs by reducing the systemic toxicity to healthy tissue (Figure 1). Free-drug impurities present in the formulated drug product, despite rigorous purification steps, can reduce the effective therapeutic window. Assessment of trace impurities is required to ensure a safe and efficacious drug product. Current UV-based detection methods offer limited sensitivity and dynamic range for detecting trace free-drug impurities below the recommended threshold.

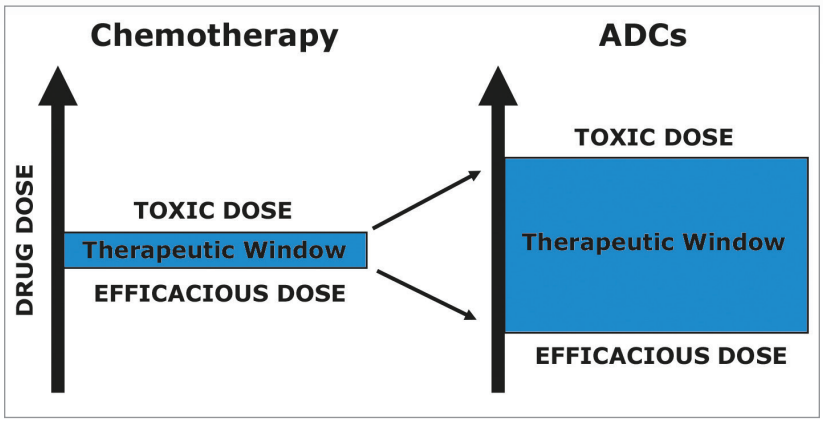

Figure 1. An illustrative concept of modality impact on the therapeutic window in the treatment of diseases using highly cytotoxic drugs.

As potentially more potent drug candidates for ADCs are identified, efforts to expand the therapeutic window will require efficient techniques with improved sensitivity and dynamic range in the detection of trace free-drug impurities to ensure safe and efficacious products are brought to market.

THE SOLUTION

Mass spectrometry (MS)-based techniques are increasingly being deployed in industry as part of a sound pharmaceutical quality system for improving product quality and product knowledge. The enhanced sensitivity and specificity of MS-based techniques make them ideal in the detection of trace-level impurities in formulated ADC drug product. To demonstrate this, a serial dilution of a quenched ADC surrogate drug molecule (NAc-linker-DSEA) based on monomethyl auristatin E was performed and analyzed using an ACQUITY UPLC® H-Class Bio System configured with both

UV (ACQUITY TUV) and MS detectors (ACQUITY QDa) in-line. Reversed-phase Liquid Chromatography (RPLC) was performed for the analysis using the CORTECS® 2.1 × 50 mm, 2.7 µm superficially porous high resolution column. Selected ion recording (SIR) of the most abundant charge state ($[M+2H]^{+2}$) was used for method assessment. As shown in Figure 2, the incorporation of mass detection extended the sensitivity for free-drug impurity detection two orders of magnitude beyond traditional UV-based detection with an LOQ of 0.33 ng/mL (1.65 pg on-column). This represents a 250-fold improvement in sensitivity compared to UV-based results (LOQ=85 ng/mL) for the same impurity. This is of particular importance given that the concentration of the same compound was determined to be 7.2 ng/mL in a post-purified ADC sample of modest concentration (1.94 mg/mL) and injection volume (10 µL). Detection of drug species at these levels is not possible with UV-based detection alone highlighting the utility of MS in extending detection limits of free-drug impurities for improved safety and efficacy of drug products.

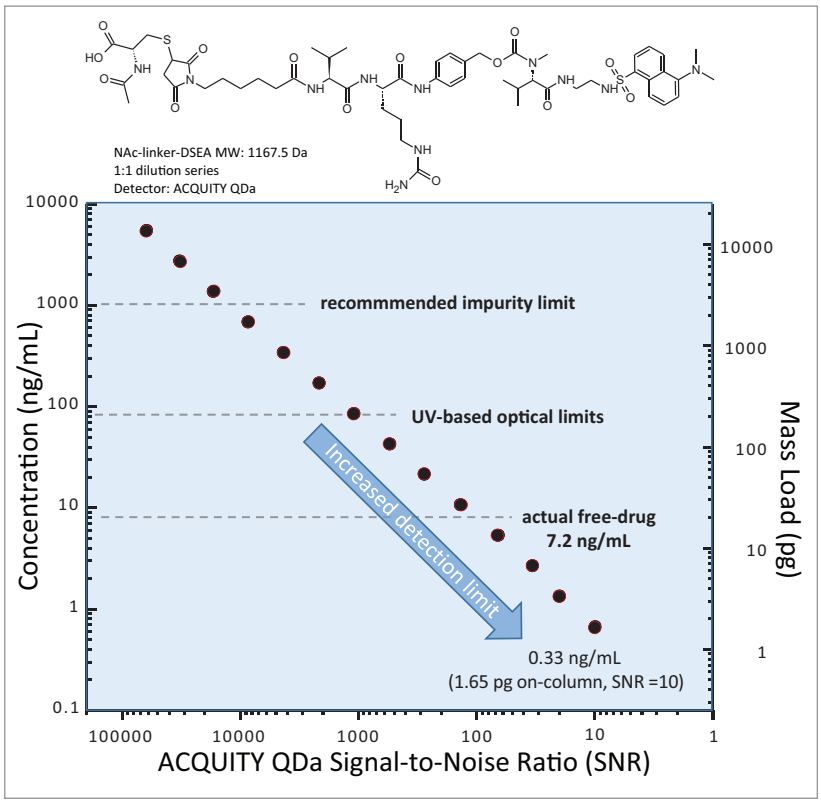

Figure 2. A 1:1 serial dilution series of Nac-linker-DSEA was evaluated to assess detector performance in impurity assays using an ACQUITY UPLC H-Class Bio System configured with an ACQUITY QDa in-line following UV detection. The dynamic range was extended over two orders of magnitude using MS-based detection with an LOQ of 0.33 ng/mL which represents a 250-fold improvement in sensitivity.

SUMMARY

As potentially more potent drug candidates for ADCs are identified, efforts to expand the therapeutic window will require efficient techniques with improved sensitivity and dynamic range in the detection of trace-level impurities. The ACQUITY QDa Mass Detector provides a robust and scalable solution for increasing dynamic range and extending detection limits beyond UV-based assays to ensure product safety and efficacy.

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