Functional Stability of Injectable Trastuzumab following Temperature Excursion or Exposure to Light

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Introduction

The availability of monoclonal antibody products such as trastuzumab in a subcutaneous injectable format has brought benefits to both patients and clinics by avoiding lengthy treatments involved for intravenous infusions. To aid spreading through the subcutaneous milieu and increase bioavailability of the active pharmaceutical ingredient the enzyme hyaluronidase is added to these products as an active excipient.

Results

Temperature excursion was modelled by heating the samples to 55ºC for 7.5 minutes. These are stress conditions intended to degrade the product. This treatment resulted in a greater than four-fold loss of measured hyaluronidase activity. In contrast there was negligible effect on the function of the monoclonal antibody as measured by its ability to inhibit growth of cancer cells *(figure 1)*. Similarly, exposure to 10 and 15 Joules of UVB light resulted in a marked and progressive loss of hyaluronidase activity, whereas there was negligible effect on trastuzumab cellular activity *(figure 2)*. Finally, exposure to D65 light (a recognised standard for outdoor daylight) followed the same pattern with 1500 megalux halving the activity of hyaluronidase and negligible effect on trastuzumab cellular activity *(figure 3)*. Subcutaneous mobility of trastuzumab showed a marked loss in heated samples which correlates with the loss of hyaluronidase activity *(figure 4)*.

Figure 1.

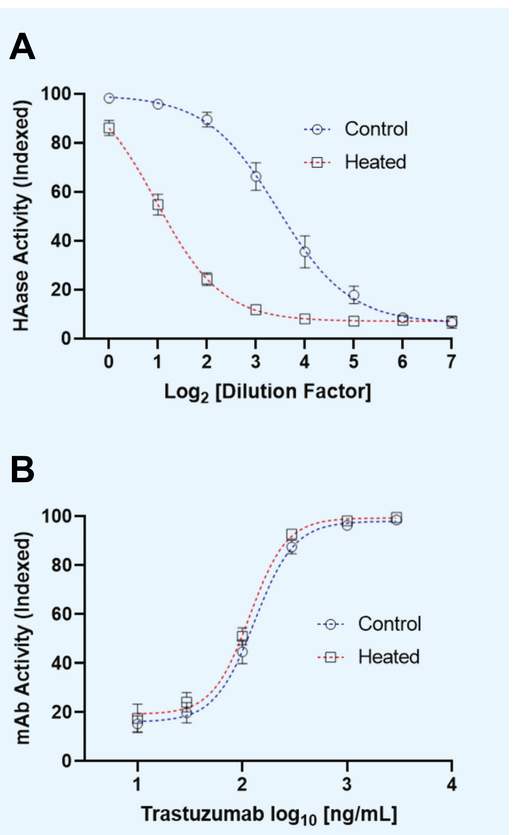

Figure 1. Functional activity of injectable herceptin ingredients following temperature excursion. Drug samples were heat stressed (55ºC for 7.5 minutes) and the functional activity of A) the hyaluronidase enzyme excipient and B) the monoclonal antibody cellular activity, were measured. Data shown are indexed against maximum function measured and are mean with standard deviation of six replicate measurements.

Figure 2.

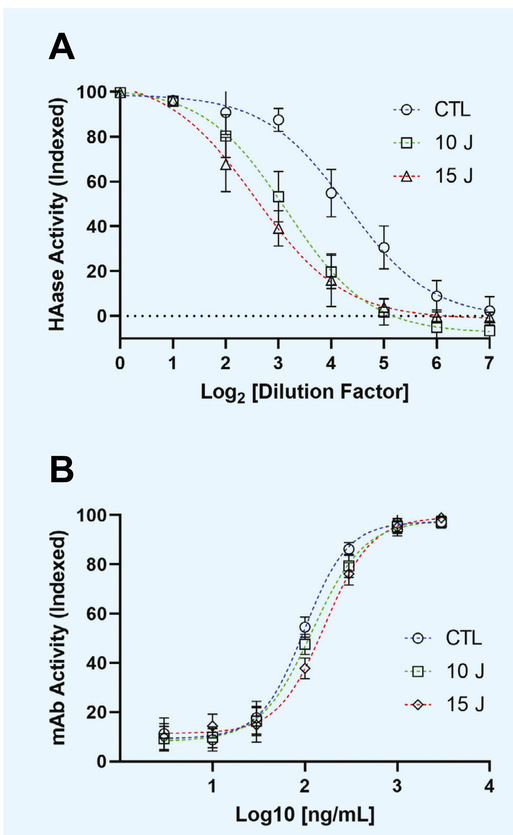

Figure 2. Functional activity of injectable herceptin ingredients following exposure to UVB. Drug samples were exposed to UVB light (control, 10 J and 15 J) and the functional activity of A) the hyaluronidase enzyme excipient and B) the monoclonal antibody cellular activity, were measured. Data shown are indexed against maximum function measured and are mean with standard deviation of six replicate measurements.

Figure 3.

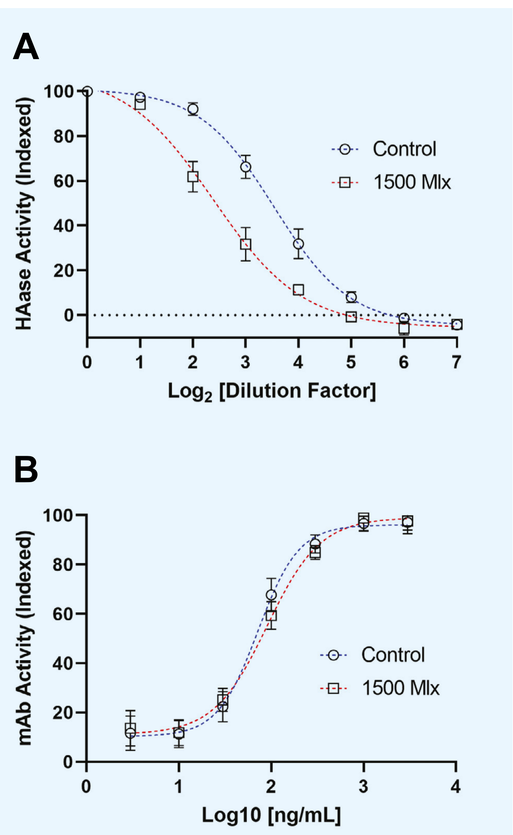

Figure 3. Functional activity of injectable herceptin ingredients following exposure to D65 light. Drug samples were exposed to D65 light (control and 1500 Mlx) and the functional activity of **A)** the hyaluronidase enzyme excipient and **B)** the monoclonal antibody cellular activity, were measured. Data shown are indexed against maximum function measured and are mean with standard deviation of six replicate measurements.

Figure 4.

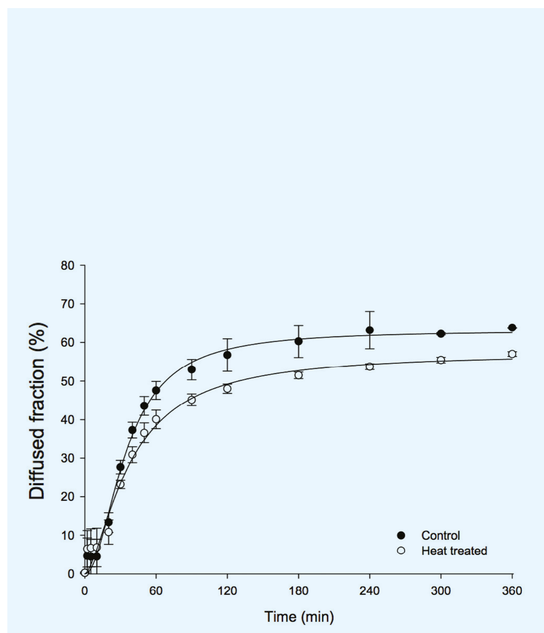

Figure 4. Subcutaneous mobility of trastuzumab following temperature excursion. Drug samples were heat stressed and mobility of trastuzumab was measured using a SCISSOR instrument. The diffused fraction of trastuzumab was measured by SEC-HPLC. Data shown are mean with standard deviation of three replicate measurements.

Aims:

To better understand the impact of excursions from normal storage conditions on injectable trastuzumab a study was performed to investigate the functional stability of both the monoclonal antibody and enzyme excipient components following exposure to stress conditions including heat and light. We also examined the impact of temperature excursion on mobility of the API in a model of the subcutaneous environment.

Methods:

Samples of trastuzumab (Herceptin 600 mg solution for injection) [1] were either stored refrigerated (5ºC ± 3ºC) and protected from light (control), or exposed to either heat, or one of two different types of light, UVB or D65. Controlled doses of light exposure were delivered using an Opsytec BS-02 irradiation chamber. Serial dilutions of the drug were prepared, and hyaluronidase activity was measured in a microplate-based assay. Trastuzumab functional activity was tested in an assay that measured the growth of BT474 breast cancer cells. Subcutaneous mobility of trastuzumab was modelled using a SCISSOR (Sirius Analytical Instruments) and the diffused fraction of trastuzumab was measured measured by HPLC and calculated using previously described methods [2].

Discussion:

Temperature excursions during transport or storage of pharmaceutical products are not rare events. EudraLex volume 4 annex 16 gives guidelines on handling unexpected deviations and states that batch release requires assessment of the potential impact of the deviation on quality, safety or efficacy of the batch(es) concerned, with the conclusion that the impact is negligible [3]. The data here demonstrates that for injectable trastuzumab the functional activity of the active enzyme excipient is less stable than the monoclonal antibody cellular activity following excursion from normal storage conditions in terms of either heat or light. Therefore, it is important to test the function of both the enzyme excipient and the active pharmaceutical ingredient when assessing the impact of unexpected deviations [4]. Whether this is true for other injectable oncology therapies such as rituximab would require further investigation.

References

[1] The Electronic Medicines Compendium. [Internet]. (2021) Herceptin 600 mg Solution for Injection in Vial. Available from: https://www.medicines.org.uk/emc/product/1227/smpc#PRODUCTINFO

[2] Eddington ND, Marroum P, Uppoor R, Hussain A and Augsburger L. (1998) Development and internal validation of an in vitro-in vivo correlation for a hydrophilic metoprolol tartrate extended release tablet formulation. Pharmaceutical Research. 15(3):466

[3] European Commision Directorate-General for Health and Food Safety. [Internet]. (2015) EudraLex Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use Annex 16: Certification by a Qualified Person and Batch Release. Available from: https://ec.europa.eu/health/sites/default/files/files/eudralex/vol-4/v4_an16_201510_en.pdf

[4] NHS Pharmaceutical Quality Assurance Committee. [Internet]. (2020) A Standard Protocol for Deriving and Assessment of Stability Part 2-Aseptic Preparations (Biopharmaceuticals). Available from: https://www.sps.nhs.uk/wp-content/uploads/2020/08/Stability-Part-2-Biopharmaceuticals-v4-Aug-2020.pdf