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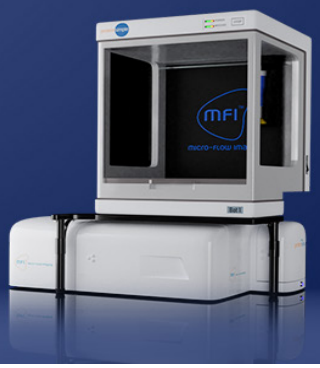
APPLICATION NOTE

EVALUATING THE STABILITY OF THERAPEUTIC BIOSIMILAR MONOCLONAL ANTIBODIES WITH MICRO-FLOW IMAGING



REVEAL THE WORLD OF SUBVISIBLE PARTICLES WITH MICRO-FLOW IMAGING

Biosimilars are a class of molecules that are identical to innovator drugs by amino acid sequence, and represent an attractive opportunity, particularly when those innovator drugs go off patent. To ensure similar quality, efficacy, and safety, certain critical quality attributes (CQA) must be assessed. One of these CQAs is the presence of subvisible particles in biopharmaceutical products. Particulate contamination, including protein aggregates, can have an effect on drug safety and efficacy, and characterization of subvisible particles can be an indicator of product and formulation stability.[1]

Micro-Flow Imaging (MFI™) from ProteinSimple directly images subvisible particles, collecting in-depth morphological data for each individual particle. With its powerful Image Analysis software, MFI can distinguish between subvisible particle types, such as protein aggregates, oil droplets, and air bubbles, thereby providing insight into the pharmacopeial particle definitions to bring a product to market. It's more sensitive than traditional methods like Light Obscuration (LO) and Manual Microscopy, and the Bot1 autosampler automates the analysis of up to 90 samples in a batch, saving on time and costs.

In this App Note, we show how we use MFI to evaluate the stability of two therapeutic monoclonal antibodies, trastuzumab and bevacizumab, and their biosimilars.

MATERIALS AND METHODS

All samples were analyzed with an MFI 5200.

The monoclonal antibodies and their respective innovator and biosimilar drug products tested in this study are listed in TABLE 1. The innovators were manufactured by Roche and the biosimilars were manufactured by Intas Pharmaceuticals.

To compare the stability of the biosimilars to the innovators, three different tests were performed: real time, accelerated, and stress. The real time test was used as a control for all the time points of the accelerated and stress tests. The storage conditions and time periods for each test are listed in TABLE 2. Both the innovator and biosimilar were analyzed at each time point simultaneously.

NO.	PRODUCT	INNOVATOR	BIOSIMILAR
1	Trastuzumab	Herclon (Roche)	Eleftha (Intas)
2	Bevacizumab	Avastin (Roche)	Bevtas (Intas)

TABLE 1. Drug products tested in this study. Water was used as a blank negative control. Herclon is also known as Herceptin.

TEST	TEMPERATURE	TIME POINT
Real Time	5 °C ± 3 °C	All time points
Accelerated	25 °C ± 2 °C	1st Day
		7th Day
		14th Day
		21st Day
Stress	40 °C ± 2 °C	1st Day
		3rd Day
		7th Day

Table 2. Storage conditions and time points for the real time, accelerated and stress tests performed in this study. At each time point, the instrument was verified with 5 µm and 10 µm bead standards before analyzing samples.

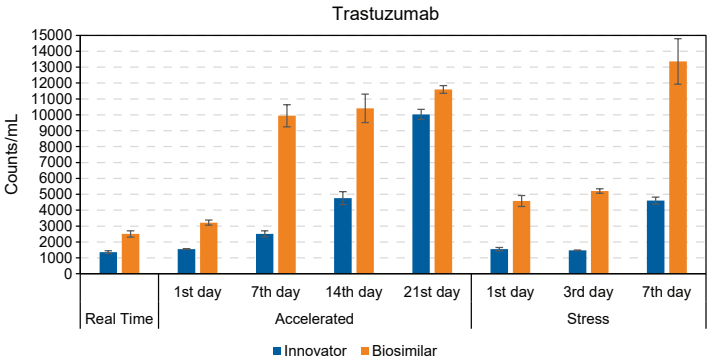


FIGURE 1. Particle formation of trastuzumab innovator and biosimilar during real time, accelerated, and stress tests. Shown are mean values with error bars representing the standard deviations of two replicates.

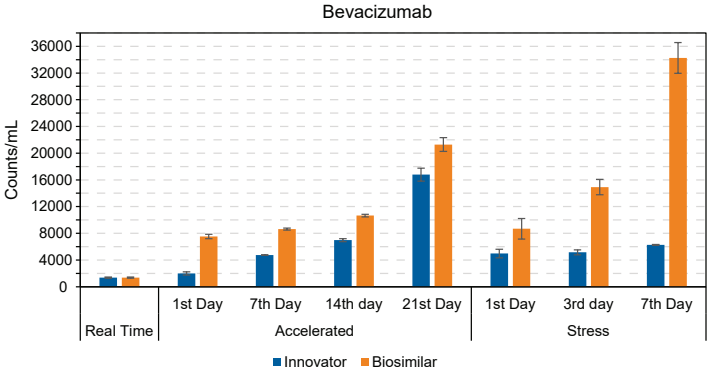


FIGURE 2. Particle formation of bevacizumab innovator and biosimilar during real time, accelerated, and stress tests. Shown are mean values with error bars representing the standard deviations of two replicates.

RESULTS

With the three tests described in the Materials and Methods, we evaluated the stability of trastuzumab and bevacizumab, two commercially successful monoclonal antibodies. Trastuzumab is used to treat HER2-positive breast cancer. It was approved by the FDA in September 1998, and several biosimilars have already been approved.[2] Bevacizumab is used for the treatment of several cancers including glioblastoma and colorectal cancer, and a biosimilar is available for therapeutic use.[3] Here, we compare the stability of both the innovator and one biosimilar for each drug product. The stability was determined by the formation of subvisible particles over time in the three tests (real time, accelerated and stress test), where the real time test was used as the reference (control) test.

This analysis showed that the trastuzumab biosimilar formed approximately 3 times more subvisible particles than the innovator for the entirety of the stress test and most of the accelerated test, except for the 21st day (**FIGURE 1**). Even at 2-8 °C (real time test), the biosimilar contained more subvisible particles than the innovator. For bevacizumab, while the number of subvisible particles in the real time test were equal, slightly more subvisible particles for the biosimilar sample were formed during the accelerated test (**FIGURE 2**). During the stress test, however, a major increase in subvisible particles for the biosimilar sample was observed on the 7th day (**FIGURE 2**), with about 6 times as many subvisible particles forming than the innovator. Collectively, these results indicate that the biosimilar of both drug products is less stable than the innovator.

Next, we evaluated the size of subvisible particles that formed during each test. For trastuzumab, the equivalent circular diameters (ECD) of particles formed were predominately in the 2-4 µm range in both the accelerated and stress test, and particles in the 5-9 µm range were second most common (**FIGURE 3**). Larger particles >25 µm were observed in the accelerated tests for both the innovator and biosimilar by the 21st day. Likewise, for bevacizumab, particle formation in the 2-4 µm range were most common for the accelerated and stress studies, and particles in the 5-9 µm range were second most common (**FIGURE 3**). Overall, counts/mL decreased with increasing particle size, with the exception of particles in the 25-39 µm range during the accelerated tests.

In general, the particles observed were highly heterogeneous and fibrous in morphology with both dense and translucent qualities. Particles were not necessarily spherical in shape and varied in intensity. All of these properties are consistent with particles of protein aggregates.[4] A representative image of particles from day 7 of each test are shown in **FIGURE 4**. Particles as large as 89.3 µm were observed on day 7 of the stress test.

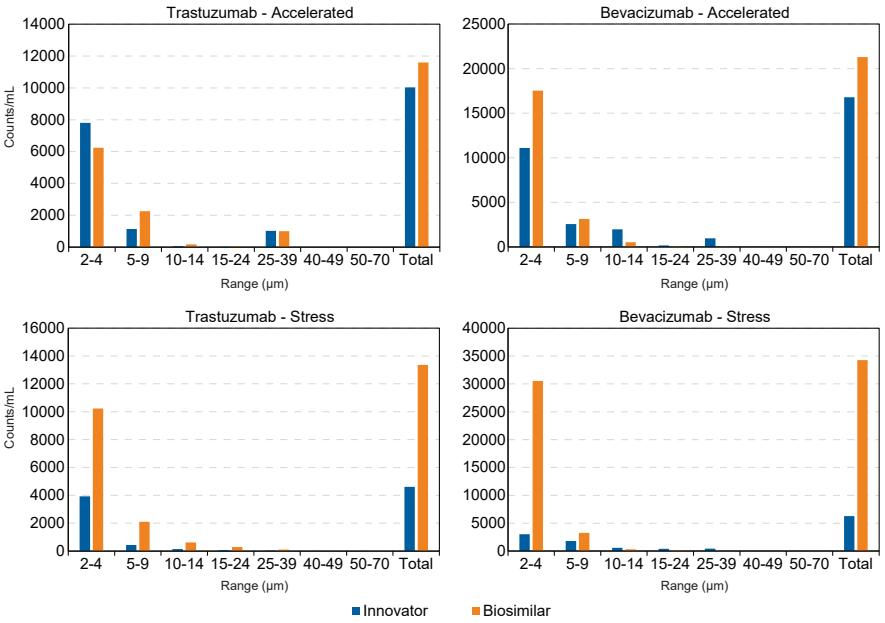


FIGURE 3. Particle sizes formed during accelerated (top) and stress (bottom) tests for trastuzumab (left) and bevacizumab (right) innovator (blue) and biosimilar (orange). Data was taken from the last day of each test (the 21st day of the accelerated test and the 7th day of the stress test).

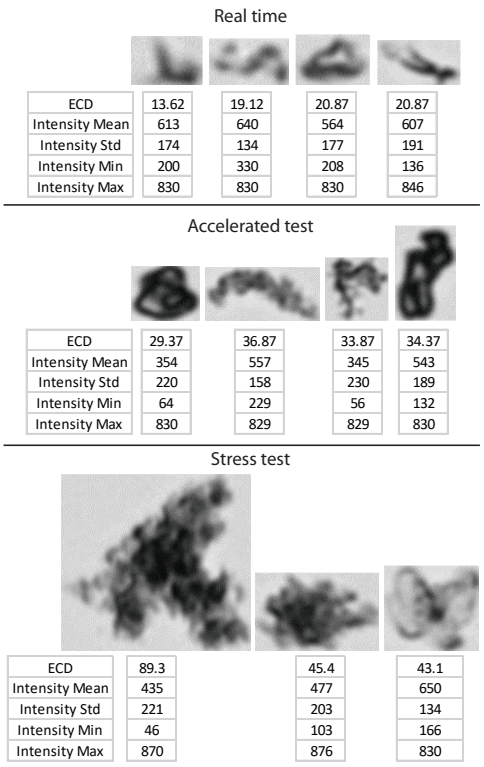


FIGURE 4. Representative images of particles observed at day 7 from real time, accelerated and stress tests. ECD and intensity parameters are shown below each particle image.

CONCLUSION

Although biosimilars are amino-acid copy drugs of innovators, even small differences in bioprocess parameters may lead to large differences in the ultimate quality of the drug product.[1] For example, the results shown here demonstrate that biosimilars of trastuzumab and bevacizumab are less stable than their innovator counterparts as measured by the formation of subvisible particles overtime in response to temperature. Based on the data presented here, storage for just one day at 25 °C is sufficient to dramatically increase particle count formations for both trastuzumab and bevacizumab. This is critical information that impacts the storage limitations and quality of these drug products.

As demonstrated here, monoclonal antibodies that are unstable tend to form protein aggregates that are subvisible particles with translucent properties. These types of particles can be very difficult to detect with LO, which is less sensitive to particles whose refractive index closely matches the surrounding matrix.[5] By contrast, MFI can directly image protein aggregates with high sensitivity, many of which would otherwise go undetected. Furthermore, the direct imaging of each subvisible particle combined with MFI Image Analysis software provides valuable information on particle morphology, including ECD, intensity standard deviation, and circularity. This enables the software to automatically distinguish between protein and non-protein particles. Taken together, MFI is a powerful solution for particulate matter analysis of drug products.

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