BSTL
experts in parenteral chemical & biological drug stability testing

Towards extending the shelf life of Aflibercept (Eylea®) in a pre-filled 1mL syringe presentation for up to 28 days at 2-8°C protected from light (PFL) – Delivering the QIPP challenge.



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Introduction

Aflibercept (EYLEA®) is used to treat the wet form of Age Related Macular Degeneration (AMD) [1]. Aflibercept belongs to the pharmacological class of anti-VEGF drugs and is a recombinant protein created by fusing: the second Ig domain of human VEGFR1, the third Ig domain of human VEGFR2, and the constant region of human IgG1 (figure 1a).

Aflibercept inhibits the binding and activation of VEGF receptors (VEGFR) by selectively binding to VEGF with high affinity. In blocking the interaction between VEGF and its native VEGF receptor Aflibercept acts as a VEGF trap and inhibits blood vessel growth. Other anti-VEGF drugs currently used as treatments for wet AMD include Ranibizumab (Lucentis®), Pegaptanib sodium (Macugen) and Bevacizumab (off label Avastin by Genentech). Each drug works in a different way to reduce blood vessel growth.

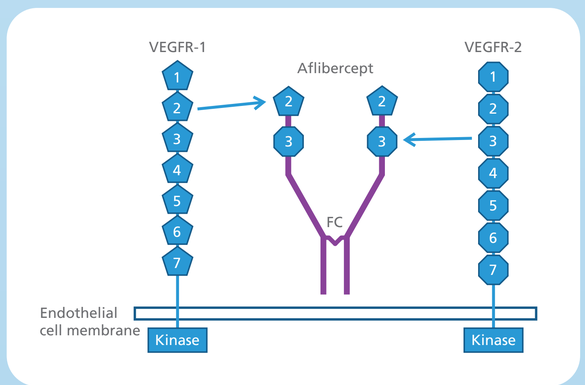


Figure 1a. Figure shows the similarity of the structure of Aflibercept and binding domains related to the human VEGFR-1 and VEGFR-2 receptors. These none identical binding domains provide for the high binding affinity of Aflibercept compared with other VEGF trap drug products.

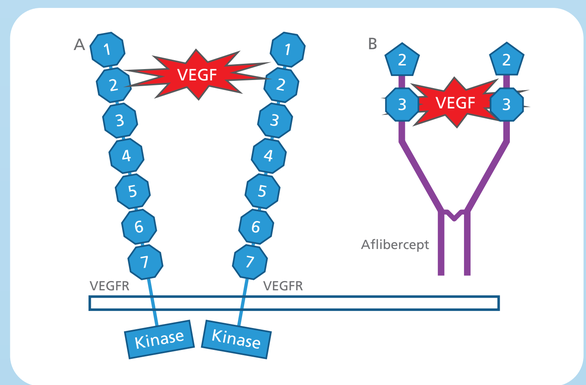


Figure 1b. The figure shows the mode of action of Aflibercept as it selectively binds to VEGF through two non identical binding domains and competes with binding of VEGF to its natural target receptor VEGFR hence "trapping" the VEGF.

Aflibercept exhibits superior binding affinity to these other VEGF blockers and has a reduced requirement for the number of injections in a treatment year compared to Ranibizumab [2,3].

Supplied in a glass vial as a RTA solution Aflibercept has to be prepared in a 1mL syringe for administration to the patient. Significant cost savings can therefore be made if Aflibercept can be prepared in advance in a pre-filled syringe using the total vial content. This requires a validated shelf life extension to be performed involving a significant R&D effort to overcome the various technical challenges in making a stability assessment of a biopharmaceutical drug product such as Aflibercept.

Background to the Study Approach

- Previously published scientific literature suggests that with certain biopharmaceutical products protein aggregation can occur when stored in a pre-filled syringe containing silicone oil as lubricant [4-7]. In this study up to three separate techniques were selected to monitor sub visible particulates within the presentations over an extended period of 28 days when stored 2-8°C PFL: light microscopy; light obscuration (LO); and Nano particle Tracking Analysis (NTA) based on dynamic light scattering. NTA is a novel technique as applied to the study of sub visible particulates in biopharmaceutical products that is gaining significant credibility and publications in this field [8].
- As the mode of action of Aflibercept is well understood and can be assessed by a binding assay, we designed an appropriate picogram sensitivity ELISA to assess the biological potency of Aflibercept within the pre-filled syringes under test. This presented a number of technical challenges due to post translational modification of the fusion protein giving rise to high non specific binding which had to be overcome in the method validation. A typical binding curve for a control solution is shown in figure 2 covering a dynamic range of 0-6ng protein in the microwell. Detection was achieved at 450nm using Ultra TMB substrate.

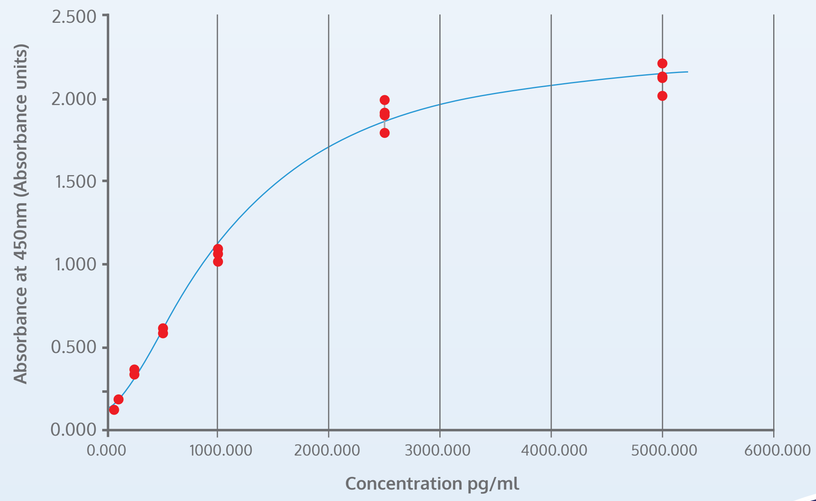


Figure 2. Figure showing a typical ELISA binding curve for Aflibercept (freshly prepared) over a concentration range of 0-6ng/mL using Ultra-TMB substrate for Horseradish Peroxidase (HRP). Detection at a wavelength of 450nm.

Applied Techniques for In-Use Physicochemical and Biological Stability Assessment of Aflibercept in a Pre-filled Syringe

An orthogonal approach including novel micro/nano analytical techniques was developed and validated to provide assurance of both the physicochemical properties and biological potency of Aflibercept following storage at 2-8°C PFL for up to 28 days. **This included:**

- **Visual inspection** – used to monitor the **appearance** of the solution using a fluorescent light magnifier to detect visible particles (>100 micron) and detect changes in colour and clarity.
- **Sub visible particle analysis** – low volume light obscuration (LO) was performed to measure particle size and number in the range 1-100 micron covering an extended length scale as required by the USP for ophthalmic injectable products.
- **pH determination** – the **pH** was measured with a micro glass combination electrode to monitor pH changes at a reduced volume of ~150 microlitres.
- **BioAnalyzer Lab on a Chip Analysis** – a miniaturised scale Sodium Dodecyl Sulphate-Polyacrylamide Gel Capillary Electrophoresis (CE-SDS) was performed with on chip fluorescence detection under both – **(a) reducing conditions** using dithiothreitol (DTT) allowing electrophoretic separation of the heavy and light chains and determination of fragment loss, and **(b) non-reducing conditions** to determine the intact protein molecular mass including presence of a 20kDa PEG moiety present as a post translation addition.
- **Size-Exclusion Chromatography (SEC)-HPLC** – this was used for the detection and quantification of mainly high molecular weight degradation products such as **protein aggregates** (e.g. dimers, up to higher order aggregates) but is also capable of detecting lower molecular weight **degradation products**.
- **Nanoparticle Tracking Analysis (NTA)** – this was used to monitor changes in the protein formulation on a Nanometer length scale up to 2 microns in the sub visible domain as an early warning indicator for protein aggregation and instability (figure 3).
- **Enzyme Linked Immunosorbent Assay (ELISA)** – developed using bespoke anti-Aflibercept conjugated Horse Radish Peroxidase (HRP) detection antibodies to quantify changes in the binding affinity of the Aflibercept following extended storage (figure 2).

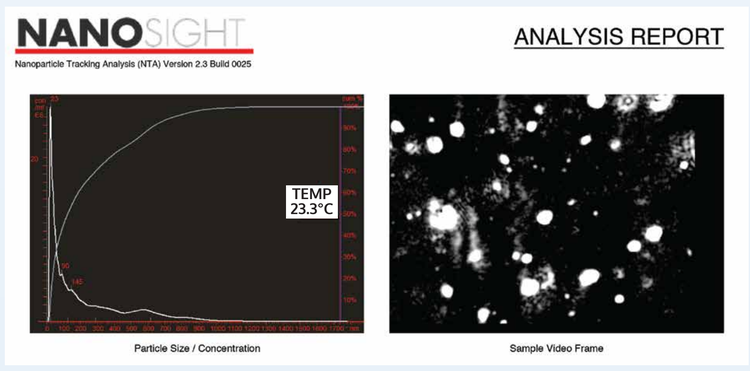


Figure 3. Typical output file from the Nanosight NTA software for Aflibercept following storage in a pre-filled syringe 2-8°C PFL. Figure 3a shows the particle size distribution from 20 to 2000nm (mode particle size 23nm). Figure 3b shows the video tracks of scattered light (not particles) detected in a field of view.

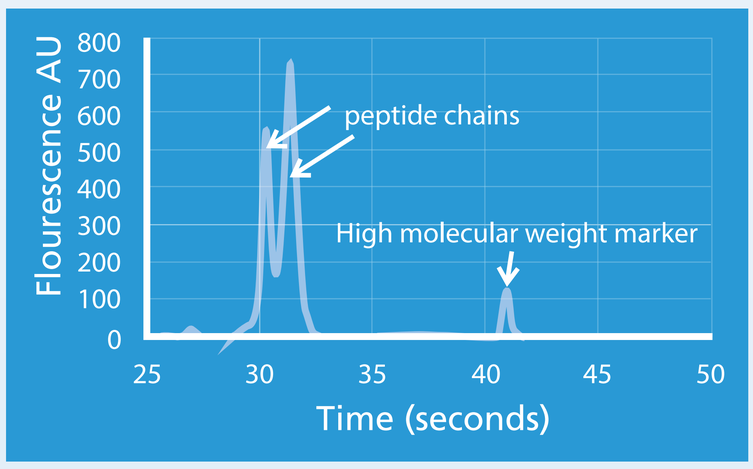




Figure 4. Typical Electropherogram following SDS-CE separation and analysis of Aflibercept under denaturing and reducing conditions using the BioAnalyzer Lab on a Chip system. Data shown for Aflibercept control presentation on Day 0.

Discussion of Data

The NTA approach enabled Aflibercept presentations to be assessed using small sample volumes for particulates ranging from 20-2000nm without sample dilution. The data shown in figure 3 shows a typical polydisperse population of particles with a mode size of 23nm. In the test samples analysed following storage < 10% of all particles had a mean hydrodynamic radius >800nm and none >1 micron. NTA was used to compare and quantify changes in the particle size distribution in test samples with control samples taken directly from a fresh vial on the day of test. Importantly this study did not find evidence for the formation of protein aggregates.

The ELISA development is beyond the scope of this poster and proved significantly more challenging than with previous monoclonal antibody studies not only in respect to initial high levels of non-specific binding. Development of a highly repeatable assay was the subject of significant R&D effort.

The application of the BioAnalyzer was also novel and provided insights in to the integrity of the Aflibercept molecule including its retention of 20kDa Polyethylene glycol (PEG) post translational modification. The effect of the pegylation is reported to have a direct effect on the mobility of the molecule under the conditions of separation [9]. This was found to be the case under non reducing conditions giving an increased apparent size. The advantages of using a Lab on a Chip capillary electrophoretic separation device included: reduced sample preparation, superior resolution and very high sensitivity of detection through on chip fluorescence.

Conclusions

The case study presented demonstrates that the application of appropriate orthogonal physicochemical and bioanalytical techniques are required in conjunction with an understanding of published scientific literature and its interpretation when assessing stability of a biopharmaceutical drug product outside of the original container. Furthermore, a thorough understanding of the mode of action of the drug being assessed is prerequisite to designing a suitable biological assay for stability and potency assessment. In the case of Aflibercept the data could support the preparation and use of a pre-filled syringe presentation with an extended shelf life beyond the typical 24 hours following first puncture of the drug vial.

The Quality, Innovation, Productivity and Prevention (QIPP) programme is a large-scale programme developed by the Department of Health to drive forward quality improvements in NHS care at the same time as making efficiency savings. Extended "In Use" stability data allows a route to the advanced central preparation of Aflibercept in pre-filled syringes on a campaign basis within a pharmacy aseptic facility and concomitant cost savings to be realised.

References

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To discuss purchase of a license for the complete 28 day stability data for Eylea in a pre-filled 1mL syringe please contact sales@biopharmatesting.co.uk
Or make a request through a BSTL representative. Or visit our website www.biopharmatesting.co.uk

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