Evaluation of size-based distribution of drug and excipient in Amphotericin B liposomal formulation

Desiree Van Hautea[a], Wenlei Jiang[b], Thilak Mudalige[a]

[a] *Arkansas Laboratory, Office of Regulatory Affairs, US Food and Drug Administration, Jefferson, AR 72079, United States*

[b] *Office of Research and Standards, Office of Generic Drugs, Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, MD 20993, United States*

Conventional quantification of drug content in the liposome formulation involves the breakdown of bulk liposomes, which ignored details on the distribution of active pharmaceutical ingredient (API) and excipients in liposomes with different sizes. The objective of this study is to develop an analytical method which can separate the liposomes into different sizes and obtain information of the drug and excipient distribution in the different sized liposomes. We developed an asymmetric flow–field flow fractionation (AF4) method for size-based separation of AmbiSome, Amphotericin B liposomal formulation and a high-performance liquid chromatography ultraviolet-visible and charged aerosol detection (HPLC-UV-CAD) method for simultaneous quantitation of the API (Amphotericin B) and the lipid excipients [1,2-Distearoyl-sn-glycero-3-phosphoglycerol (DSPG), hydrogenated soy phosphatidylcholine (HSPC), and cholesterol]. The measured drug and individual lipid content in the bulk liposome formulation was consistent with the drug product labeling. Liposomes were separated using AFFF into eleven size fractions and the distribution of liposomes particles sizes of each fraction were measured with nanoparticle tracking analysis. However, the drug to total lipid ratios in fractioned liposomes increased from 0.1 to 0.45 when the average liposome size increased from 75 nm to 124 nm, while the lipid composition remained constant throughout the fractioned size range (cholesterol:DSPG, 0.7 and HSPC:DSPG, 0.3). These study results suggested that, for formulations with Amphotericin B in liposomes, the drug to lipid ratio increases with the size of the liposomes.