Validation of an ELISA for Quantitation of the Lysosomal Protein Saposin C following Protein-lipid Nanovesicle BXQ350 Delivery to Nonhuman Primates

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BXQ-350 is a nanovesicle formulation of the lysosomal protein Saposin C and phospholipid dioleoylphosphatidylserine (DOPS) (SapC-DOPS) that selectively induces apoptosis in tumor cells, using a novel mechanism of action. A sandwich ELISA method for determination of SapC concentrations in K2EDTA-containing cynomolgus monkey plasma was developed and validated to support bioanalytical work on GLP-compliant toxicology studies. The calibration standard curve ranged from 0.43 to 12.4 ng/mL. Monkey plasma spiked with BXQ350 at the concentration of the lower limit of quantitation (LLOQ), low, mid and high assay ranges, and upper limit of quantitation (ULOQ) was used as quality control (QC) samples to evaluate overall performance of the assay including intra-day and inter-day accuracy and precision. Dilutional linearity was established for spiked QC samples diluted from 1:1,000 (minimal required dilution) to 1:50,000. No differences were observed between standard curve samples prepared in 0.1% monkey plasma and those prepared in buffer, suggesting that plasma was a suitable matrix for the assay. Although the assay was vulnerable to artifacts in spiked QC samples, selectivity was demonstrated using both spiked and unspiked samples at the ULOQ and LLOQ for the assay. Stability of SapC within stock solutions of BXQ350 was established for 193 days of storage at -20 °C. Stability of SapC in QC plasma samples was determined for 2 hours of short-term storage at room temperature and in incurred study samples for 229 days of long-term storage at -20 °C. In conclusion, a suitable validated ELISA method for quantitation of SapC in monkey plasma was developed.