



## Measurement of oligonucleotides in preclinical and clinical testing

We are developing a therapeutic antisense oligonucleotide for the treatment of liver cancer. It is formulated in a liposomal vehicle and administered by infusion, and we would like to measure intact oligonucleotide in plasma and urine samples to support both nonclinical and clinical studies. Is there a particular bioanalytical technique that you would recommend?

As oligonucleotides are manufactured by chemical synthesis using commercially available, automated synthesizers and organic reagents, they are considered more similar to new chemical entities (NCEs; small-molecule drugs) than biologics despite their large sizes (molecular weights generally 6-7 kDa and above).

Our recommendation would be to develop a hybridization-based enzyme-linked immunosorbent assay (ELISA) for measurement of your drug in order to fully establish the pharmacokinetic (PK) profile following administration.

This method uses complementary oligonucleotide probes for specific Watson-Crick base-pairing between the oligonucleotide analyte and designed probe, resulting in minimal matrix effects and exceptional sensitivity over conventional chromatographic techniques.

Selection of assay format is key, and given that your requirements are for the measurement of intact parent oligonucleotide, we would work to develop a method that is highly selective for the parent compound whilst excluding potentially interfering truncated sequences within the assay. Our team can design the appropriate complementary probes for this reason, while aiming to achieve the highest assay sensitivity.

More selective, tighter binding to the complementary probes can be achieved by introducing modified nucleic acids, such as locked nucleic acids (LNA) and peptide nucleic acids (PNA), at intermittent positions within the complementary probe strand. We have been able to realize sensitivity levels in the low pg/mL using this approach.

Given that your compound is formulated within a liposome, we would require both drug substance and drug product forms prior to initiating method development experiments to assess whether the assay conditions (e.g., suitable detergent, sonication or thermal disruption) can liberate the oligonucleotide from formulation. We would prepare quality control (QC) samples in the appropriate matrix with the encapsulated drug to best reflect the study samples. These samples would be interpolated to a calibration curve prepared with free oligonucleotide in order to ensure that the oligonucleotide can be quantitatively measured. Since very little sample volume is required (25 to 100  $\mu$ L of sample generally utilized for analysis in duplicate), this method is easily amenable to multiple species matrices, with no need for sample extraction.

Also owing to the specific binding between the analyte and its complementary probe, these assays can be applied to different species with little or no change in the procedure. This, in turn, minimizes the cost and time associated with developing separate bioanalytical methods in different species as your drug progresses from a preclinical to clinical setting.