Gene-Based RNA and DNA Therapeutics Pharmaceutical Development Support

Fast TAT | Fluorescence Hybridization | HRMS | LC/MS/MS | Oligonucleotides

pyxant LABS

BIOANALYTICAL CONTRACT RESEARCH

Therapies development involving oligonucleotides may require high-resolution mass spectrometry (HRMS), and/or hybridization-HPLC-fluorescence techniques, which we are implementing at Pyxant Labs, in addition to LC/MS/MS.

LC/MS/MS

Advantages for using LC/MS/MS are specificity, speed, and cost. LC/MS/MS has improved specificity over traditional detection methods due to the ability to monitor the transition of a precursor ion to a specific product ion. This transition can be uniquely optimized to the molecule of interest. Typically, LC/MS/MS can be used for quantitation over three orders of magnitude and low ng/mL to µg/mL detection levels are common. Additionally, it has the ability to quantitate co-eluting peaks based on mass transition ions, allowing for increased speed over traditional light-based LC detection methods.

LC/MS/MS is an established technology accepted by regulatory agencies and most reviewers are familiar with its application. Software is standardized throughout the industry, is 21 CFR Part 11 compliant, and has become the gold standard for bioanalytical quantitation.

Disadvantages with LC/MS/MS include the mass range of the instrument typically cannot detect molecules with masses greater than 20 kDa, and it is limited to a mass resolution of no better than 0.1 Da. Mass resolution deteriorates substantially as molecular masses increase.

High-Resolution Mass Spectrometry

The main advantage of HRMS is better mass resolution and superior selectivity with analytes which cannot be resolved via LC/MS/MS. HRMS allows resolution of analytes to the nearest 0.0001 Da, or better, even for molecules exceeding 100,000 Da. By measuring exact mass, minor changes in the mass of the molecule of interest can be distinguished from endogenous interferences, which may be native to a sample matrix. HRMS also allows for both qualitative and quantitative applications. This allows for the quantitation of known molecules, while simultaneously offering the ability to identify biomarkers or metabolites during a single analytical run.

The disadvantage of HRMS is limited dynamic range and higher instrument cost and maintenance when compared to quadrupole LC/MS/MS platforms.

Hybridization-HPLC-Fluorescence

Hybridization-HPLC-fluorescence assays hybridize a fluorescent probe onto a target molecule and detect the fluorescent-tagged product. The benefit of this technique over ELISA is the ability to differentiate among metabolites, interferences, and the analyte of interest utilizing chromatographic separation. Additionally, this hybridization assay often facilitates lower detection limits (LLOQ) than LC/MS/MS or HRMS assays, most particularly for oligonucleotides.

Disadvantages include the design of fluorescent probes, background noise (specificity), and long run times (>20 minutes), which precludes using this technology for quick turnaround studies. For oligonucleotides, this technique is often limited to a maximum of 20-mers.