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A Universal Method for the Quantitation of Multiple Oligonucleotides from Plasma by MFLC-MS/MS

Bioanalysis of oligonucleotides by HPLC-MS/MS in support of regulatory submission has not been widely adopted. Several obstacles such as lack of sensitivity, adduct formation and analytical instrument dedication are barriers that many laboratories are unwilling to challenge. Traditional ELISA and qPCR bioanalysis of oligonucleotides may not have the selectivity to accurately quantify the therapeutic oligonucleotide from complicated biological matrix. HPLC-FL methods require a probe design that increases the method development time and cost of the assay. Additionally, the probe is not suitable for oligonucleotides smaller than 20 nucleotides. HPLC-MS/MS analysis is an alternative approach that provides better selectivity, increased dynamic range and the flexibility to analyze a vast array of oligonucleotides from biological matrix. In order to increase the sensitivity of the method, analysis was conducted using microflow HPLC (MFLC) conditioned for oligonucleotide analysis thus reducing adduct formation. The microflow system is on a portable cart that allows the scientist the ability to transport the pumps to any mass spectrometer available without allocating an entire LC-MS/MS system for oligonucleotide analysis. A universal MFLC and solid phase extraction (SPE) method was developed to extract and analyze five oligonucleotides from human plasma.