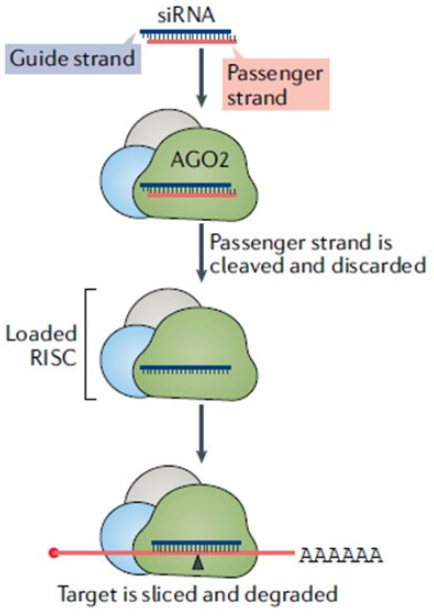
BIOANALYSIS

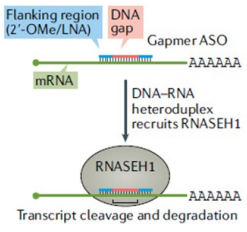
OLIGONUCLEOTIDE THERAPEUTICS

Cutting-Edge Omics For Your Research

Oligonucleotides (ONs) are single-stranded or double-stranded drugs consisting of 12-30 nucleotides, including antisense oligonucleotides (ASOs), small interference RNA (siRNA) and aptamers. Their main mechanism of action is to bind to the target RNA through the principle of base complementary pairing, degrade the target RNA by endogenous nuclease, or regulate the RNA splicing and translation process through the mechanism of steric configuration blocking ribosome, so as to achieve the therapeutic purpose of the disease. In preclinical pharmacokinetic studies, the concentration of oligonucleotide drugs in vivo over time needs to be assessed by precise quantitative means. Oligonucleotide drugs have high water solubility, multiple negative charges, a wide range of intra-plasma concentrations, and low concentrations in the post-plasma distribution phase, which pose a great challenge for bioanalysis. Commonly used techniques include liquid chromatography-tandem mass spectrometry (LC-MS/MS) and Hybridization based LC fluorescence (LC-FLD).

Bioanalysis of oligonucleotides is generally performed by liquid-liquid extraction (LLE) or solid-phase extraction (SPE). LLE is the most common way to extract oligonucleotides, and the commonly used extractant is phenol-chloroform. Tissue extracts have strong matrix effects and low extraction recoveries, and SPE is generally used. oligonucleotides are acidic and strongly polar compounds, which are difficult to retain on general chromatographic columns. The main chromatographic methods for oligonucleotides are ion-pair reversed-phase chromatography (IP-RPLC), ion exchange chromatography (IEC), and hydrophilic interaction chromatography (HILIC). Among them, ion-pair reversed-phase chromatography (IPRPLC) is the most commonly used method, which mainly uses ion-pair reagents. The principle is that positively charged ion-pair reagents will form ion-pairs with negatively charged oligonucleotide phosphate backbones in solution, which reduces the net charge of the oligonucleotide and increases its hydrophobicity, and promotes the retention of the oligonucleotide in the ion-pair reversed-phase chromatography. As oligonucleotides contain multiple nucleotide groups, they undergo deprotonation in the ESI source, forming multiply charged ions in the negative ion mode, as well as readily binding metal ions, thus dispersing the mass spectral signal. In addition, with the increase in molecular weight of the oligonucleotide, there is a severe loss of detection response. Therefore, for such compounds it is necessary to optimize the mass spectrometry conditions to improve the multiple charge distribution and to reduce the formation of additive ions in the mass spectrometry analysis of the substance.





Oligonucleotide-mediated gene regulatory mechanisms

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