LC/MS/MS VS. LC/HRMS

Identifying and quantifying oligonucleotides

Oligonucleotides (OGNTs) are short, single- or double-stranded DNA or RNA molecules that play diverse roles in molecular biology, as they yield approaches to target diseases at the molecular level. Two advanced analytical techniques used in mass spectrometry to identify and quantify OGNTs are LC/MS/MS and LC/HRMS. Considerations for choosing the right approach for your drug development project will depend on what instrument you have, where you want to go, and how much information you want to collect in a single injection.

CONSIDERATIONS FOR CHOOSING YOUR APPROACH

CHROMATOGRAPHY	IONIZATION	FULL SCAN VS FRAGMENTATION
• Peak shape • OGNT retention • Carryover	• Formation of multiple charge states • Formation of metal adducts	• Specificity of the formed fragments • Intensity of the fragment vs intensity of the parent ion

LC/MS/MS VS LC/HRMS

| LC/MS/MS	LC/HRMS
• Better sensitivity • Easier to maintain • Better software • More readily available • Higher familiarity	• Sensitivity is adequate for most PK programs • Collects a lot of data that can be used downstream from analysis (data mining) • No tuning required • Larger mass detection window

LC/MS/MS

LC/MS/MS combines liquid chromatography (LC) with tandem mass spectrometry (MS/MS) to analyze complex mixtures of molecules. Because this method is highly sensitive and capable of quantifying molecules in complex samples, it is often used for protein/peptide analysis
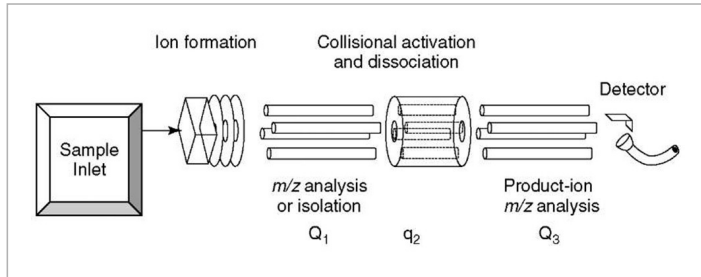
LC/MS/MS can optimize dwell times to improve sensitivity. During method development it's important to monitor multiple charge states to determine which will afford better selectivity and sensitivity. Also consider different ion pairing reagents to drive the charge envelope into the mass range of the instrument.

LC/HRMS

LC/HRMS combines liquid chromatography with high-resolution mass spectrometry, allowing for more accurate determination of molecule mass of ions. Because ions are sorted based on their mass-to-charge ratio, even the slightest differences in mass can be detected, making this method useful for applications that require accurate mass determination.
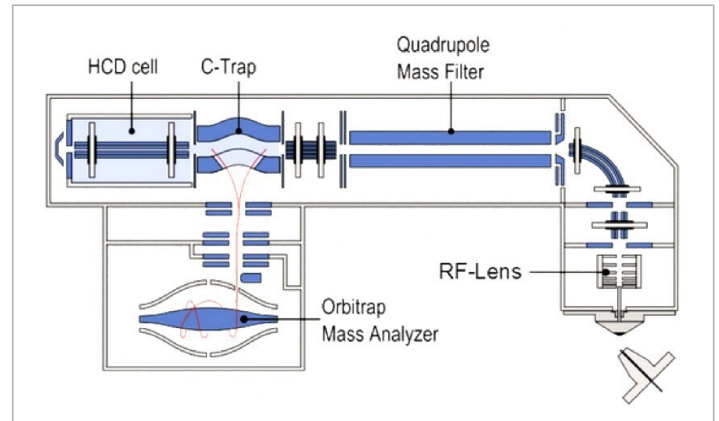
Two different data acquisition modes in LC/HRMS are TSIM (targeted selection ion monitoring) and Full Scan. TSIM will drop overall data points per monitored targeted mass points, and Full Scan offers data points for all masses and enables the ability to mine data post-acquisition. It's recommended to use TSIM when you need to identify specific compounds with high sensitivity and selectivity, and Full Scan when you're interested in identifying a wide range of compounds.

Both LC/MS/MS and LC/HRMS are powerful tools that yield detailed insights into the composition and structure of molecules for biotechnology and pharmaceuticals. Contact us to learn more.