Bioanalytical monitoring of gene therapy trials: methodologies for PK profiling of oligonucleotides

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ABSTRACT

- Understanding the chemistry and pharmacology of allele-specific oligonucleotides (ASO) and small interfering RNAs (siRNA) is advancing
- Application of oligonucleotides in gene and enzyme-replacing therapy for treatment of rare and orphan diseases is becoming a more attractive commercial target for pharma and biotech
- Bioanalytical monitoring of gene therapy toxicology studies and clinical trials needs precise methodologies for pharmacokinetics assessments
- Accessibility to high-resolution mass spectrometry is fundamental for accurate and sensitive determination of PK profiling of oligonucleotide drugs
- High recoveries can be achieved with two-dimensional chromatography allowing accurate quantification in the pg/mL for plasma (low ng/mL for tissue) within a broad dynamic range
- Our standard UPLC-HRMS workflow delivers qualitative and quantitative data with high throughput without compromising data quality (run time of 4 minutes injection-to-injection)
- Metabolite identification can be obtained on a similar chromatographic platform, requiring a 20-30 minutes runtime (less throughput but higher molecular detail).
- PK of oligonucleotide drugs can be efficiently and reliably addressed through implementation of our optimized UPLC-HRMS and UPLC-MS/MS workflows while generating valuable metabolic information in different matrices and species, from in vitro and preclinical studies to clinical development.

**NUCLEIC ACID THERAPEUTICS**

<table>
<thead>
<tr>
<th>Antisense Oligonucleotides</th>
<th>Synthetic mRNA</th>
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<tbody>
<tr>
<td>ssRNA</td>
<td>siRNA</td>
</tr>
<tr>
<td>4,000-6,000 MW</td>
<td>13,000-16,000 MW</td>
</tr>
<tr>
<td>14-20 nucleotides, single strand</td>
<td>1,500-2,000 nucleotides</td>
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<tr>
<td>Translation attenuation; RNAaseH based degradation</td>
<td>Gene expression</td>
</tr>
<tr>
<td>Often chemically modified</td>
<td>Typically un-modified</td>
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</tbody>
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**LC-MS**

- Mass <25 base (ss)
- In matrix, SPE, LLE
- Un-amplified
- 1-10 ng/mL LLOQ
- ISR
- Excellent specificity

**BIOL ANALYTICAL PLATFORMS**

- Hybridization ELISA or LCFLD
- qPCR

**Bioanalytical challenges**

- Full PK profiling should address:
  - Plasma
  - In vitro and in vivo metabolism
  - Tissue distribution
  - Potential renal excretion
  - Highly charged drug molecule
  - Cation adducts can severely reduce the signal of the ion of interest, decrease sensitivity, hard to troubleshoot
  - High-sensitivity, accurate and selective methods
  - GLP compliant quantification for IND/ClinTA-enabling studies
  - High sample throughput
  - Stable calibration
  - Uniform resolution over a large mass range to accurately determine the mass of multiply charged parents and metabolites
  - Robust ion-source requiring minimal cleaning
  - Degradation by Exo- and Endonucleases
  - Quantitation needed for parent drug and metabolites
  - No specific regulatory guidelines

**Solutions**

- Advancements in MS platform:
  - Quantitative → ID and Quantitative
  - TripleToF® 5600 → TripleToF® 6600
  - LLOQ <5 ng/mL → 100 pg/mL
  - Rapid WAX SPE extraction (Clarity, NH2/DAC/ACN)
  - Dynamic range 10^11
  - Excellent calibration
  - Working resolution: 35K to 40K
  - Narrow mass extraction window (50-75 mDa)
  - High throughput: 3x96 well plate batch per day (4 min injection-to-injection run time)
  - Fits validation acceptance criteria for small molecules and chromatographic methods
  - GLP compliant quantitation per latest FDA BMV guidance

**Case study – Validation of PK methods for the determination of a therapeutic si-RNA oligonucleotide in cynomolgus monkey Plasma**

**Assays for plasma PK profiling and tissue distribution**

**Chromatograms and full scan HRMS spectra - Run time: 4 minutes injection to injection**

**Calibration curve parameters for anti-sense and sense strands in Monkey plasma**

**Extracted ion chromatogram for profiling and metabolite identification in plasma and kidney tissues**

**In-house developed software solution to assign ions to metabolites**

**Degradation by Exo- and Endonucleases**

**Metabolite identification**

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- Stable calibration
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