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

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ABSTRACT

- Understanding of the chemistry and pharmacology of allele-specific oligonucleotides (ASO) and small interfering RNA's (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

Antisense Oligonucleotides	siRNA	Synthetic mRNA
ssRNA	dsRNA	ssRNA
4,000-6,000 MW	13,000-16,000 MW	450,000-600,000 MW
14-20 nucleotides, single strand	22-27 nucleotides double strand	1,500-2,000 nucleotides
Translation attenuation; RNAseH based degradation	RISC based degradation	Gene expression
Often chemically modified	Also chemically modified	Typically un-modified

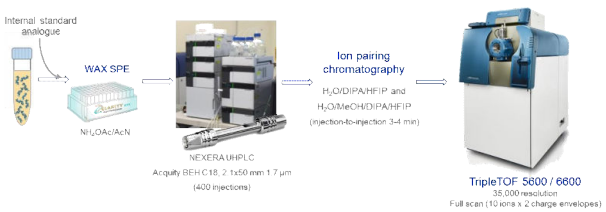
BIOANALYTICAL PLATFORMS

LC-MS	Hybridization ELISA or LC/FLD	qPCR
Mass <25 base (ss)	Specific hybridization <30 bases (ds)	Specific hybridization >60 bases
In matrix, SPE, LLE	In matrix, SPE	Extraction
Un-amplified	Enzymatic signal amplification	Exponential signal amplification
1 -10 ng/mL LLOQ	1 ng/mL LLOQ	50 copies LLOQ
ISR	ISR	-
Excellent specificity	Good specificity Background interference possible	Excellent specificity

Assays for plasma PK profiling and tissue distribution

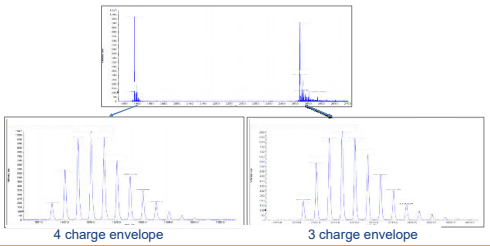
Bioanalytical challenges

- 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 quantitation for IND/CTA-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



High Resolution Mass Spectrum of a typical siRNA (resolution 35k)

Full Scan Quantitation: summing the response from accurate mass of 10 most intense ions (5 each from the isotopic envelope of the $[M]^{4-}$ and $[M]^{3-}$ charged states) for antisense, antisense IS, sense, sense IS, = '40-in-1'

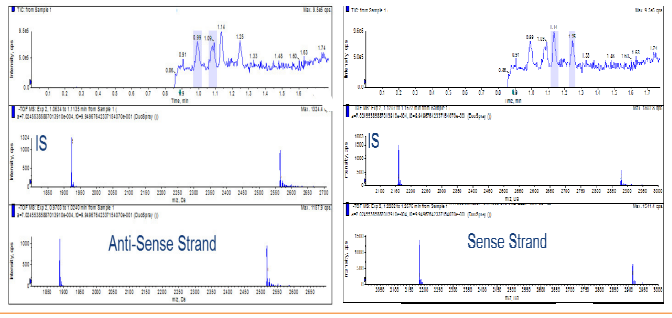


Solutions

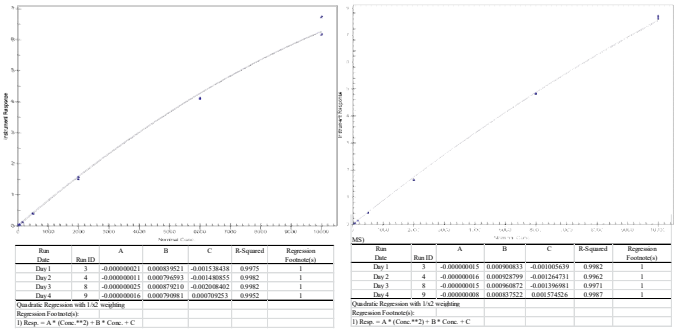
- Advancements in MS platform:
  - **LC-MS/MS** (2003) → **LC-HRMS** (2014)
  - Quantitative → ID and Quantitative
  - TripleToF® 5600 → TripleToF® 6600
  - **LLOQ <5 ng/mL** → **100 pg/mL**
- Rapid WAX SPE extraction (Clarity, NH_4OAC/ACN)
- Dynamic range 10^3
- Excellent calibration
- Working resolution: 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
- Assays developed and validated in multiple matrices
  - Plasma
  - Excreta (urine, feces)
  - Tissues (adrenal glands, brain, fetus, heart, jejunum, kidney, liver, lung, pancreas, spleen, testes, thyroid, thymus)
- Oligo-conjugates:
  - GalNAc, lipid, cholesterol

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

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



Run Date	Run ID	A	B	C	R-Squared	Regression Footnote(s)
Day 1	3	-0.000000021	0.000839521	-0.001538438	0.9975	1
Day 2	4	-0.000000011	0.000796593	-0.001480855	0.9982	1
Day 3	8	-0.000000025	0.000879210	-0.002008402	0.9982	1
Day 4	9	-0.000000016	0.000790981	0.000709253	0.9952	1

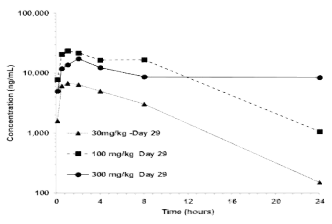
Quadratic Regression with 1/x2 weighting
Regression Footnote(s):
1) Resp. = A * (Conc. **2) + B * Conc. + C

Run Date	Run ID	A	B	C	R-Squared	Regression Footnote(s)
Day 1	3	-0.000000015	0.000900833	-0.001005639	0.9982	1
Day 2	4	-0.000000016	0.000928799	-0.001264731	0.9962	1
Day 3	8	-0.000000015	0.000960872	-0.001396981	0.9971	1
Day 4	9	-0.000000008	0.000837522	0.000574526	0.9987	1

Quadratic Regression with 1/x2 weighting
Regression Footnote(s):
1) Resp. = A * (Conc. **2) + B * Conc. + C

Plasma concentration of full length oligonucleotide in monkey plasma

sub-cutaneous administration of 30, 100, and 300 mg/kg dose



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

30 min chromatographic run for identification; followed by a 4 min run for quantification based on XIC

In-house developed software solution to assign ions to metabolites