CASE STUDY

From divergent calibration curves to 100% sample acceptance: ASO Quantitation in CNS Tissues

Aliri BIOANALYSIS

How Aliri Bioanalysis resolved divergent calibration curves and matrix suppression to deliver a LC-MS/MS method for a 17-mer antisense oligonucleotide across spinal cord and brain tissue.

MODALITY	MATRICES	PLATFORM	OUTCOME
17-mer 2'-MOE ASO	NHP CSF, NHP/mouse brain, NHP/mouse spinal cord	Q-Exactive PRM, DNAPac RP column	**100% acceptance across all tissue types**

This work highlights several tangible benefits for sponsors seeking robust, regulatory-ready bioanalytical support for CNS-targeted oligonucleotide programs:

- **Tissue-specific science, not a one-size-fits-all protocol:** The work demonstrated that even within the same species, different tissues (mouse brain vs. mouse spinal cord) required different extraction methods, a nuance that is easy to miss and costly to overlook in CNS drug development.
- **Faster path to reliable data:** By diagnosing the matrix effect directly (via plasma-only vs. tissue-spiked comparisons) rather than guessing, Aliri avoided wasted development cycles chasing the wrong root cause.
- **Directly applicable to CNS-targeted ASO and oligonucleotide programs:** Sponsors developing therapeutics that require brain or spinal cord tissue PK/PD data, a common requirement for neurology-focused oligonucleotide drugs, gain a proven, tissue-matched extraction framework rather than a single generic method forced onto every matrix.

The Challenge

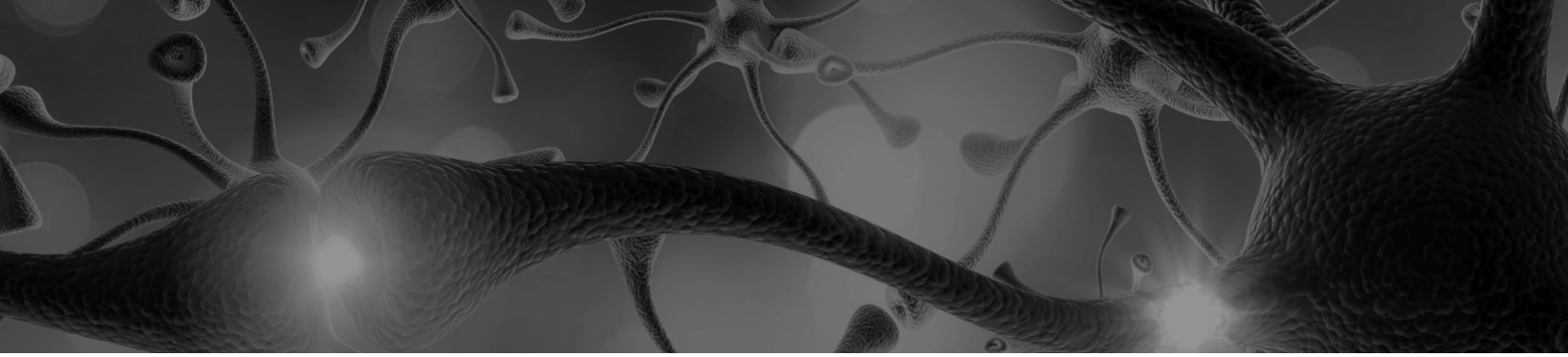
A sponsor developing a 17-mer 2'-MOE antisense oligonucleotide (ASO) needed a qualified bioanalytical method to quantify drug levels not only in cerebrospinal fluid, but in brain and spinal cord tissue homogenate matrices that are notoriously difficult for oligonucleotide LC-MS work. While the extraction method that worked well for NHP CSF and brain used a Clarity OTX solid-phase extraction (SPE) plate and produced an acceptable quadratic regression across 20.0–5,000 ng/mL, that same approach broke down when applied to new matrices.

Extending the method to mouse brain, mouse spinal cord, and NHP spinal cord homogenate surfaced a cluster of problems that would stop many labs in their tracks: significant divergence above 3,000 ng/mL, two distinct calibration curve slopes appearing in the same run, and a non-linear, quadratic instrument response. Left unresolved, these issues would have compromised data quality precisely in the tissues most relevant to a CNS-targeted therapeutic, the matrices the sponsor most needed reliable data from.

The Approach

Rather than treating the divergence as an instrument or suppression problem by default, Aliri scientists systematically ruled out the more common explanations first. Instrumentation testing confirmed no ion suppression was affecting the samples at the retention times of interest. The team then compared plasma-only ASO standards against plasma spiked with tissue homogenate, and found the two produced measurably different results, confirming a true, tissue-specific matrix effect rather than an instrument artifact.

This distinction mattered: the fix had to happen at the extraction chemistry level, not the LC-MS method, and that a single extraction protocol was unlikely to serve every tissue equally well.



Aliri evaluated two extraction chemistries in parallel, tailored to what each tissue type actually needed:

- **Liquid-liquid extraction (LLE):** Using concentrated ammonium hydroxide, phenol:chloroform:isoamyl alcohol, water, and dichloromethane to separate the ASO from the aqueous and organic layers, this approach successfully resolved the divergence problem in mouse brain homogenate, producing a clean quadratic regression (R^2 = 0.9930, %CV = 5.3) with no divergent curves or split slopes.
- **Phenyl SPE with TEAA/DIPEA chemistry:** Spinal cord tissue in both species proved more resistant. Loading onto a phenyl SPE plate with triethylammonium acetate (TEAA) buffer, eluting with 1% N,N-diisopropylethylamine (DIPEA) in methanol, and passing the eluate through a protein precipitation filter plate delivered a significant improvement in linearity, eliminated the divergence, and produced acceptable results under both linear and quadratic regression models.

Notably, when the ASO was extracted from plasma alone, without any tissue homogenate present, instrument response ratios were 10- to 30-fold higher across the calibration curve than in tissue-containing samples. This finding was itself valuable: it confirmed that the tissue matrix itself, not the extraction procedure, was suppressing signal, and it reinforced why a tissue-matched, individualized extraction strategy, rather than a single universal protocol, was the right scientific approach.

The Results

The final, optimized methods delivered strong, reproducible linearity across every matrix tested:

NHP – SPINAL CORD	MS – SPINAL CORD	MS – BRAIN
R^2 = 0.9860 %CV = 6.6%	R^2 = 0.9905 %CV = 6.6%	R^2 = 0.9903 %CV = 6.8%

These figures represent a dramatic improvement over the initial phenol-chloroform trials in spinal cord tissue, where R^2 values had been as low as 0.5239–0.8416 with %CV values exceeding 70% in the worst case, numbers that would have failed acceptance criteria and delayed the sponsor's program. Across the final validated methods, sample analysis yielded a 100% acceptance rate.

This work underscores a core principle behind Aliri's oligonucleotide bioanalysis practice: chemical modifications and matrix properties both shape how an oligonucleotide behaves during extraction and ionization, and reliable quantitation in complex tissues demands individualized, tissue-specific method development, not a default protocol applied uniformly. For sponsors advancing ASO and other oligonucleotide therapeutics into CNS tissues, that distinction is often the difference between clean, submittable data and months of troubleshooting.

Source: *Davies, B., Wilcock, B., Fang, T., Bettencourt, B., Hardcastle, C., Voelker, T. "Antisense Oligonucleotide Extraction Optimization Reveals Significant Differences Between Non-Human Primate Spinal Cord, Mouse Spinal Cord, and Mouse Brain Tissue Homogenates." ASMS 2024, Abstract #583. Aliri Bioanalysis, Salt Lake City, UT.*

Aliri
aliribio.com
USA: +1 (719) 593-1165
FRANCE: +33 (0)374 095 546