A Process of Method Development for Tissue Homogenization and Analysis to Eliminate Binding and Matrix Effects Using Surrogate Matrices

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PURPOSE

The analysis and distribution of pharmaceuticals in tissue is increasingly important in understanding the full action of drugs in vivo. Across molecular types from very small molecules to larger peptides and oligonucleotides, bioanalysis of tissues presents unique challenges inherent quantitative bioanalysis in a mixed matrix assay. In this work, homogenization methods for tissue samples are assessed for non-specific binding and analytical congruency of surrogate and tissue homogenates across species and tissue types. Generation of a broadly applicable method of sample processing has resulted in streamlined method development and provided robust conditions applicable to a variety of matrices. Assessment of processes are also described in optimizing the final method to individual analytical needs for compound types in specific tissues. Accurate and precise results in multiple tissue types across multiple species are obtained with reduced time spent in development using a baseline set of experiments and set points for optimization of parameters.

OBJECTIVES

- Describe a method of processing to mitigate binding and suppression effects in tissue homogenates
- Demonstrate surrogate curves are effective at compensating for matrix effects in multiple matrices
- Demonstrate increased workflow efficiency and acceptable assay performance using a single surrogate curve for multiple tissues

METHODS

Fit-for-purpose bioanalytical methods were generated independently to quantify a small molecule in rat tissues and oligonucleotide in monkey tissues using LC-MS/MS detection. Tissues were homogenized using a Bullet Blender homogenizer and either steel or ceramic beads. Comparison of homogenate methods were made to determine processing stability and non-specific binding and optimized into a final processing method that is tissue type and analyte specific using an established battery of tests. Common tissue types were diluted in same species plasma to eliminate binding effects and plasma surrogate control experiment is conducted to determine the acceptability of plasma quantification of the limited quantity tissue matrices. Rare tissue types were homogenized in appropriate buffer solutions. Non-specific binding and surrogate parallelism experiments were conducted to determine the optimal buffer solution and tissue weight to volume dilution ratio. Extraction methods were developed to analyte specific requirements and employ both protein precipitations for small molecule method described and SPE methodology common to various oligonucleotides

METHODS (CONTINUED)

LC-MS/MS methods are optimized for signal, mass/charge and interference contribution using a Sciex API 5000 mass spectrometer and UPLC separations using Shimadzu Nexera integrated dual system LC setup.

RESULTS:

A similar approach was used for the initial tissue assessment of both molecule classes to determine a standard set of best starting conditions and experiments for optimization of final methods. Buffer homogenate diluents and plasma were both tested and compared for NSB and accuracy of homogenate QCs against a diluent only calibration curve to conserve rare matrices. Analysis of a small molecule using the optimized method in rat liver and spleen tissues showed <4.6% CV and -5.2 to 5.1 %RE for Liver QC samples and <5.0% CV and -4.7 to 5.1 %RE for Spleen QC samples in a mixed tissue run using a plasma standard curve method derived in the optimization process. Analysis of a small molecule in rat skin homogenate was acceptable with >6.5% CV and -9.8 to 14.4% RE. Analysis of an oligonucleotide in monkey brain, liver and kidney demonstrated acceptable accuracy and precision utilizing a buffer homogenate and surrogate control matrix. Brain homogenate QC samples were analyzed with <4.3 %CV and -3.5 to 3.6% RE in a fit-for purpose assay against the surrogate curve. Liver and Kidney homogenates were run in a mixed matrix analysis with <11.3%CV and -10.0 to 11.2% RE for kidney QC samples and <7.6% CV and -5.0 to 7.4 % RE for liver QC samples in a fit-for purpose assay against the surrogate curve. The acceptability of the data across multiple tissue types demonstrates the utility of a common approach using surrogate plasma curves to quantitate tissue matrices in a single run to expedite analysis and conserve project material resources.

CONCLUSIONS:

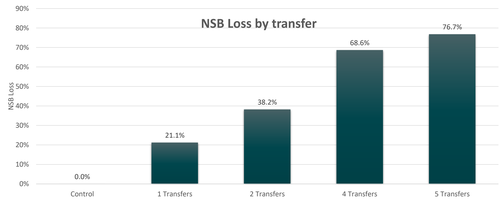
An approach was found to determine levels of non-specific binding and to mitigate matrix effects in the process of homogenization and sample prep. This approach can be broadly applied to a variety of tissue homogenates and other matrices. A workflow approach of systematic optimization experiments allows for methods to be generated quickly for a variety of molecules and drug classes. Comparison of single tissue QCs against single tissue samples using surrogate matrix standard controls for acceptance provided a more robust comparison in a fit-for-purpose analysis while conserving such rare and costly matrices as primate cerebrospinal fluid and tissues.

Non-specific binding and matrix effects in homogenized tissues samples are mitigated using optimized surrogate controls for accurate quantitation of pharmaceuticals in mixed matrix bioanalytical methods

ASSESSMENT OF BINDING EFFECTS

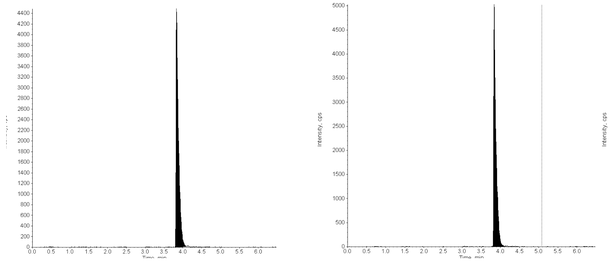
Distribution of drug in tissues can be very heterogeneous. Full validations are not needed or not possible for these matrices. A process of homogenization optimized to yield the best recovery is essential.

Transfer tests in tubes containing homogenization buffer and materials are performed to determine the level of non-specific binding to tube and beads. High levels of non-specific binding present in plot below indicated buffer was not an acceptable surrogate whereas using plasma for the homogenization diluent mitigated all binding effect to tube and beads to less than 5% loss after the 5th transfer.



SURROGATE USED FOR RARE MATRICES

Tissues for control matrix are expensive and difficult to obtain especially in non-clinical species. Rare and expensive matrices can be conserved by using fully surrogate controls, also minimizing the cost of life. Artificial CSF (aCSF) or treated buffer solutions are more readily available. Additives can be used to control NSB and are examined for ion suppression during method development. Chromatograms of an oligonucleotide at LLOQ level in aCSF(Left) and Primate CSF (right) are depicted below demonstrating peak integrity and comparable signal in the selected surrogate.



PLASMA SURROGATE / MATRIX QC A&P ASSESSMENT

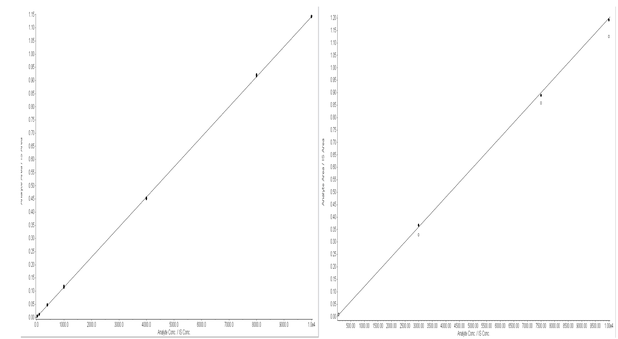
Plasma standards are used to quantify tissues homogenized in plasma to control for binding effects and mitigate suppression effects in tissue homogenates. QC samples at 10,000 ng/mL are diluted with 10x plasma for dilution effect comparisons. Both tissue types homogenized in plasm show good agreement of the data. This normalization effect and mitigation of the binding effects to the sample processing material makes plasma both an optimal diluent and surrogate for tissue analysis.

Liver Tissue	20.0 ng/mL	60.0 ng/mL	3000.0 ng/mL	7500.0 ng/mL	[a]10000.0 ng/mL	10000.0 ng/mL
Mean	21.3	61.3	3176.0	7748.9	10619.2	9820.6
S.D.	1.4	2.0	77.7	138.4	230.0	327.7
% RSD	6.6	3.3	2.4	1.8	2.2	3.3
% RE	6.5	2.2	5.9	3.3	6.2	-1.8
n	6	6	6	6	6	6

Spleen Tissue	20.0 ng/mL	60.0 ng/mL	3000.0 ng/mL	7500.0 ng/mL	[a]10000.0 ng/mL	10000.0 ng/mL
Mean	23.3	3163.6	3163.6	7602.5	10808.1	9768.5
S.D.	2.7	75.5	75.5	141.7	219.6	203.0
% RSD	11.6	2.4	2.4	1.9	2.0	2.1
% RE	16.5	5.5	5.5	1.4	8.1	-2.3
n	6	6	6	6	6	6

PLASMA SURROGATE USED IN MULTI-TISSUE ANALYSIS CONSERVE MATERIALS AND TIME

More data can be obtained in fewer analytical runs by employing mixed tissue methods. QC samples in homogenate are representative of sample and match the processing of whole tissue sections. Dilution of tissues in plasma for homogenization provides normalization across samples between tissue types. A method can be developed in plasma and employed as tissue analysis. As tissues samples are also lower in number across a study mixed tissue analysis can complete a program in shorter time with less material and instrumentation requirements. Parallelism of rat plasma (left) and rat liver homogenate (right solid) and rat spleen homogenate (right open) are shown below.



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