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CASE STUDY

Stability of ASO in Tissue Matrix

ASO concentrations in monkey and rat plasma were successfully developed and validated to support preclinical studies. Our objective was to transfer the previously developed bioanalytical method into human gut tissue. During the method transfer and pre-validation stage, all critical evaluations for selectivity, specificity, precision and accuracy, dilution linearity, and prozone were acceptable. The stability of the ASO in gut tissue at the homogenization stage was tested following three freeze/thaw cycles (Table 5A) and at room temperature (22 °C Nominal) (Table 5B) for 26.9 hours. In both evaluations, the ASO was under-recovering at both the QC1 and QC3 levels. As gut tissues were suspected to be rich in bacterial flora with high levels of nucleases, proteinase K was added during sample spiking. The addition of proteinase K did not improve the recovery in either case.

Table 5 A. Freeze/thaw stability (3 F/T Cycles) for analyte in human gut tissues (homogenate).

	Stability QC Without Proteinase K		Stability QC with Proteinase K	
	QC1	QC3	QC1	QC3
	6.938*	719.7*	1.343*	501.1*
	3.367*	380.3*	2.025*	519.5*
N	2	2	2	2
Nominal Concentration (ng/g)	37.5	1875	37.5	1875
Mean	5.152	550	1.684	510.3
SD	2.525	240	0.4827	13.02
%CV	49	43.6	28.7	2.6
%RE	-86.3	-70.7	-95.5	-72.8

Table 5 B. Analyte stability in thawed matrix stored at room temperature (22 °C nominal) for 26.9 hours in human gut tissues (homogenate).

	Stability QC Without Proteinase K		Stability QC with Proteinase K	
	QC1	QC3	QC1	QC3
	3.662*	435.2*	1.844*	445.4*
	2.494*	419.3*	1.298*	403.3*
N	2	2	2	2
Nominal Concentration (ng/g)	37.5	1875	37.5	1875
Mean	3.078	427.3	1.571	424.3
SD	0.8259	11.29	0.3861	29.71
%CV	26.8	2.6	24.6	7
%RE	-91.8	-77.2	-95.8	-77.4

*: % Accuracy Unacceptable for QCs

As the stability of the drug was impacted at the homogenization step, we concluded that sample extraction should be performed immediately following homogenization to prevent any possible ASO degradation. To confirm the time period of ASO stability in gut tissue homogenate, the drug was spiked in gut tissue homogenate at QC1 and QC3 level and tested at room temperature (22 °C nominal) for 1, 2, 3, and 4 hours. Table 6 illustrates that the drug is stable up to four hours. These results indicate that gut tissue homogenate should not be frozen/thawed; instead, the extraction should be done within four hours of the homogenization procedure.

Table 6. ASO stability at room temperature (22 °C nominal) at different time points (1 hr, 2 hr, 3 hr, and 4 hr) in human gut tissues (homogenate).

	Stability QC 1 hr		Stability QC 2 hr		Stability QC 3 hr		Stability QC 4 hr	
	QC1	QC3	QC1	QC3	QC1	QC3	QC1	QC3
	36.47	1742	34.09	1679	36.53	1571	32.11	1530
	37.09	1760	33.65	1633	37.49	1650	35.41	1500
	33.48	1617	39.48	1444	37.02	1637	32.46	1476
N	3	3	3	3	3	3	3	3
Nominal Concentration (ng/g)	37.5	1875	37.5	1875	37.5	1875	37.5	1875
Mean	35.68	1706	35.74	1585	37.01	1619	33.33	1502
SD	1.929	77.84	3.244	124.3	0.4836	42.63	1.812	26.86
%CV	5.4	4.6	9.1	7.8	1.3	2.6	5.4	1.8
%RE	-4.8	-9	-4.7	-15.4	-1.3	-13.6	-11.1	-19.9

Conclusions

New generation ASOs have better potency and bio-distribution. Thus, for concentration determination, high method sensitivity is required to achieve an accurate PK profile at the preclinical stage, which will drive decisions on clinical dosing. By increasing the sensitivity of the assay, other issues may arise, such as selectivity or metabolite interferences, and so it is even more critical to evaluate all types of interferences in the bioanalytical method during method development. Finally, since there are no ICH or FDA regulatory guidelines for the validation of ASO products, our strategy is to follow the general principles outlined in FDA 2018[8] as a starting point for method validation, where applicable.

ABOUT ALTASCIENCES

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