Bioanalytical Analysis of Sunitinib Microparticles for Ocular Administration

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Objectives

To understand the ocular distribution of sunitinib and its metabolite desethyl sunitinib from extended release microparticles in non-GLP rabbit studies through the development of a fit-for-purpose assay utilizing plasma as a single surrogate matrix for all ocular tissues.

Due to the early phase of the drug development and the scarcity of ocular matrices, typical GLP validation conditions were unnecessary, impractical and cost-prohibitive. AIT Bioscience focused on finding a surrogate matrix method that would give robust quantitation to support early development and PK assessment as well as early drug distribution and toxicology analysis.

Background

Graybug Vision has developed a sunitinib microparticle formulation as a potential treatment for wet age-related macular degeneration (wAMD). Development of this drug and dose selection requires evidence of controlled-release drug delivery to the desired ocular tissues with low systemic availability. To address this requirement, AIT Bioscience developed a fit-for-purpose dual range assay covering a quantitation range of 0.100 – 50,000 ng/mL. This method has been used successfully for over 2000 samples to support non-GLP in vivo rabbit studies. The assay demonstrates high levels of drug in ocular tissues and low to BLQ levels in aqueous humor and plasma.

Sample Preparation Method

All samples and solutions containing analytes or internal standards are protected from ambient (unfiltered) light at all times during sample extraction and injection.
Rabbit ocular tissues (cornea, iris/ciliary body, RPE/choroid, lens, sclera, conjunctiva) are homogenized prior to extraction. Tissue samples are diluted with K_2EDTA rabbit plasma based on tissue weight and homogenized with ceramic beads using a Precellys 24 – Dual homogenizer. Retina is diluted with plasma and homogenized by vortexing and sonication. Vitreous and aqueous humor are diluted with plasma and mixed well before assay.
A sample volume of 25 µL of plasma or tissue homogenate was aliquotted into a 1.2 mL 96-well plate and mixed with 50 µL internal standard solution in methanol and 50 µL 95-5 Water-NH_4OH. The plates were transferred to a Tomtec Quadra96 liquid handler, which was used to add 600 µL of ethyl acetate to each well. The plates were covered and the mixtures were vigorously shaken, vortexed to mix, and centrifuged. A 400 µL aliquot of the organic phase was transferred, evaporated to dryness under nitrogen at 35°C, and reconstituted in 200 µL of water-acetonitrile-formic acid, 80-20-0.1.

Conclusions

The method described provides for the quantitation of high local and low systemic concentrations of sunitinib in plasma and ocular tissue. This method has allowed for PK analysis and drug distribution studies to support early phase development. The use of plasma as a homogenization medium and surrogate matrix meets scientific rigor, provides ease of analysis in the lab and addresses ethical concerns of procuring scarce ocular matrices.

Instrumental Analysis Method

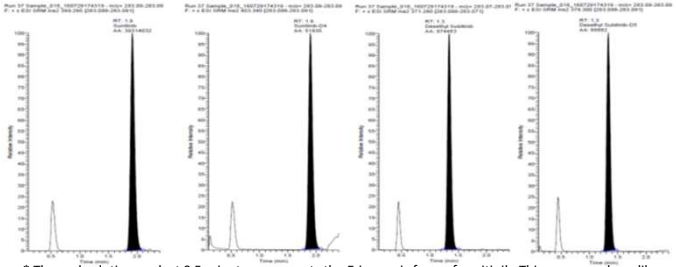
HPLC Instrumentation: Thermo Dionex UltiMate
Mass Spec: Thermo Scientific TSQ Quantiva triple quadrupole mass spectrometer; ESI
Column: Acquity BEH C18 column (2.1 x 50 mm; 1.7 µm), 30°C.
Mobile Phase A was 90:10:0.1 water:acetonitrile:formic acid.
Mobile Phase B was 10:90:0.1 water:acetonitrile:formic acid.

Time (min)	Flow Rate (mL/min)	%A	%B
0.00	0.500	80.0	20.0
1.70	0.500	80.0	20.0
1.80	0.500	40.0	60.0
3.90	0.500	40.0	60.0
4.00	0.500	80.0	20.0
6.00	0.500	80.0	20.0

SRM Table

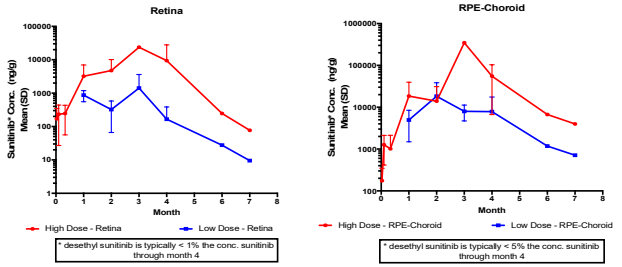
Compound	Expected Retention Time (min)	Precursor Exact Mass/Charge (m/z)	Product Observed Mass/Charge (m/z)	Charge State of Precursor Ion
Sunitinib	1.81	399.27	283.1	+1
Sunitinib-D4	1.79	403.34	283.09	+1
Desethyl Sunitinib	1.20	371.28	283.07	+1
Desethyl Sunitinib-D5	1.20	376.3	283.09	+1

Chromatograms

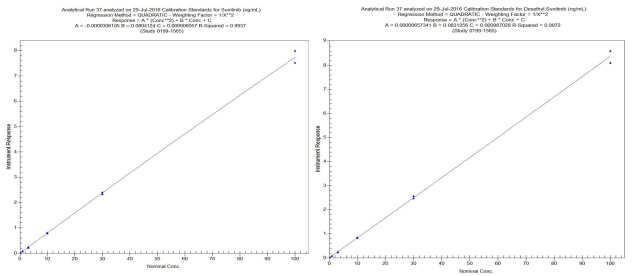


* The early eluting peak at 0.5 minutes represents the E-isomeric form of sunitinib. This compound readily isomerizes in the presence of light. Isomerization is controlled during analysis by working under LED lighting conditions and keeping 96-well plates protected from light.

PK results for ocular tissues



Regressions



Method Performance

Sunitinib	0.300 ng/mL	3.00 ng/mL	30.0 ng/mL
Mean	0.31	3.04	30.6
% CV	9.6	4.1	4.7
% Bias	3.3	1.3	2
n	24	24	24

Desethyl-sunitinib	0.300 ng/mL	3.00 ng/mL	30.0 ng/mL
Mean	0.296	3.01	30.6
% CV	7	1.7	3.2
% Bias	-1.3	0.3	2
n	24	24	24