Table 1. Total Amount of RO0503821 in Serum and Urine from Male Rats (Study D01017, SDS-PAGE Estimation)

Analysis of RO0503821 in SERUM Samples

Rat Group ID	**Serum Conc (μg/mL)**	*Vol. of Serum (mL)	Total RO0503821 in Serum (μg)	Dose (mg) RO0503821 Administered	**Percent of Dose in Serum**
30 min IV	175	10	1389	2.0	83
24 hr IV	56	9	422	2.0	25
24 hr SC	65	5	286	2.0	17

*The serum volumes were estimated as 3.0% of rat's body weight.

Analysis of RO0503821 in URINE Samples

Rat Group ID	**Urine Conc (μg/mL)**	Vol. of Urine (mL)	Total RO0503821 in Urine (μg)	Dose (mg) RO0503821 Administered (mg)	**Percent of Dose in Urine**
24 hr IV	2.75	13	35.7	2.0	1.8
24 hr SC	0.62	11	6.81	2.0	0.3

Total amount of RO0503821 in rat serum and urine: Male rats were administered 2 mg RO0503821 by IV or SC injection. Serum samples were taken at 30 minutes and at 24 hours post dose. Approximately 10 μL aliquots of a 1 to 9 dilution of serum (1 part serum + 9 parts PBS) and of undiluted urine were analyzed by SDS-PAGE (Figure 1). The percent of the dose present in the serum or urine at the two time points following IV or SC administration was estimated by scanning densitometry of the iodine stained gel.

Calculations:

Serum and urine concentration (μg/mL) = concentration of RO0503821 in sample aliquot determined by densitometry using standard curve in same gel.

Volume of serum (mL) = 3% of rat's body weight

Volume of urine (mL) = total volume excreted in 24 hour sample.

Total amount RO0503821 in serum (μg) = concentration in serum aliquot (μg/mL) x total serum volume in body (mL)

Total RO0503821 in urine (μg) = concentration in urine aliquot (μg/mL) x total volume of urine excreted in 24 hour sample period (mL).

Percent of dose in serum = (amount (μg) in serum) / (2 mg dose) x 100

Percent of dose in urine = (amount (μg) in urine) / (2 mg dose) x 100

2.6.5.9 In Vivo Metabolism

Test Article: **RO0503821-000**

Gender (M/F)/ Number of animals:	D01017: M/4 D02001: M /28
Feeding condition:	D01017: Fed D02001: Fed
Vehicle/Formulation:	D01017: PBS, pH 7.0 D02001: 10 mM sodium phosphate, 40 mM sodium sulfate, 10 mM L-methionine, 3% mannitol, 0.1% polaxamer 188 pH 6.2
Method of Administration:	D01017: subcutaneous and intravenous D02001: Intravenous
Dose (mg/kg)	D01017: ~6-11 mg/kg D02001: ~8 mg/kg

Species	Sample	Sampling Time or Period	% of Dose in Sample	Study Number	Location in CTD
Rat	Serum	30 min post IV dose	83	D01017	
	Serum	24 hr post IV dose	25		
	Urine	24 hr post IV dose	1.8		
	Serum	24 hr post SC dose	17		
	Urine	24 hr post SC dose	0.3		

Additional information:
Both studies were aimed to evaluate *in vivo* stability and tissue localization of the test compound. The results confirmed that 1) RO0503821 remained intact in serum; 2) both RO0503821 and 30 kDa PEG were excreted in urine and 3) no *in vivo* catabolism of RO0503821 prior to its passage through kidneys.