

Development and Validation of a Sensitive LC-MS/MS Method for the Quantification of SGR-1505 in Human Plasma to Support Clinical Pharmacokinetic Studies

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PURPOSE

MALT1 is a key mediator of NF-κB signaling and an emerging therapeutic target in B-cell malignancies and autoimmune diseases. SGR-1505, a potent MALT1 inhibitor, is being clinically evaluated for its therapeutic potential. The purpose of this study was to develop and validate a reliable and high-throughput LC-MS/MS method for the quantitation of SGR-1505 in human plasma (K₂EDTA) to support clinical pharmacokinetic studies. This work exemplifies the critical role of CRO-led bioanalysis in bridging early discovery and clinical development of emerging therapeutics.

OBJECTIVE

To develop and validate a sensitive and selective bioanalytical method for the quantification of SGR-1505 in human plasma (K₂EDTA) using liquid chromatography–tandem mass spectrometry (LC-MS/MS) in the range of 10.0 to 10,000 ng/mL. The method was designed to support pharmacokinetic and clinical studies by ensuring accurate and reproducible measurement of SGR-1505 concentrations across the anticipated clinical range.

METHOD

A 20.0 μL aliquot of human K₂EDTA plasma was transferred to a 96-well plate. To each well (except blanks), 50.0 μL of 400 ng/mL SGR-1505-D5 in 50:50 water/MeCN was added; blanks received 50.0 μL of 50:50 water/MeCN. After brief centrifugation (~3,000 rpm, 1 min) and vortex-mixing (~1 min), 500 μL MeOH/MeCN (50/50) was added, followed by 5 min vortex and centrifugation (~4,500 rpm, 5 min). A 50.0 μL supernatant aliquot was, diluted with 400 μL water/MeCN (90/10), vortex-mixed (~1 min), and centrifuged (~3,000 rpm, 1 min) before LC-MS/MS. Protein precipitation was chosen for its speed, simplicity, and high-throughput efficiency, using minimal sample (20.0 μL) and basic equipment. The 96-well format enables semi-automation with consistent recovery and low carryover.

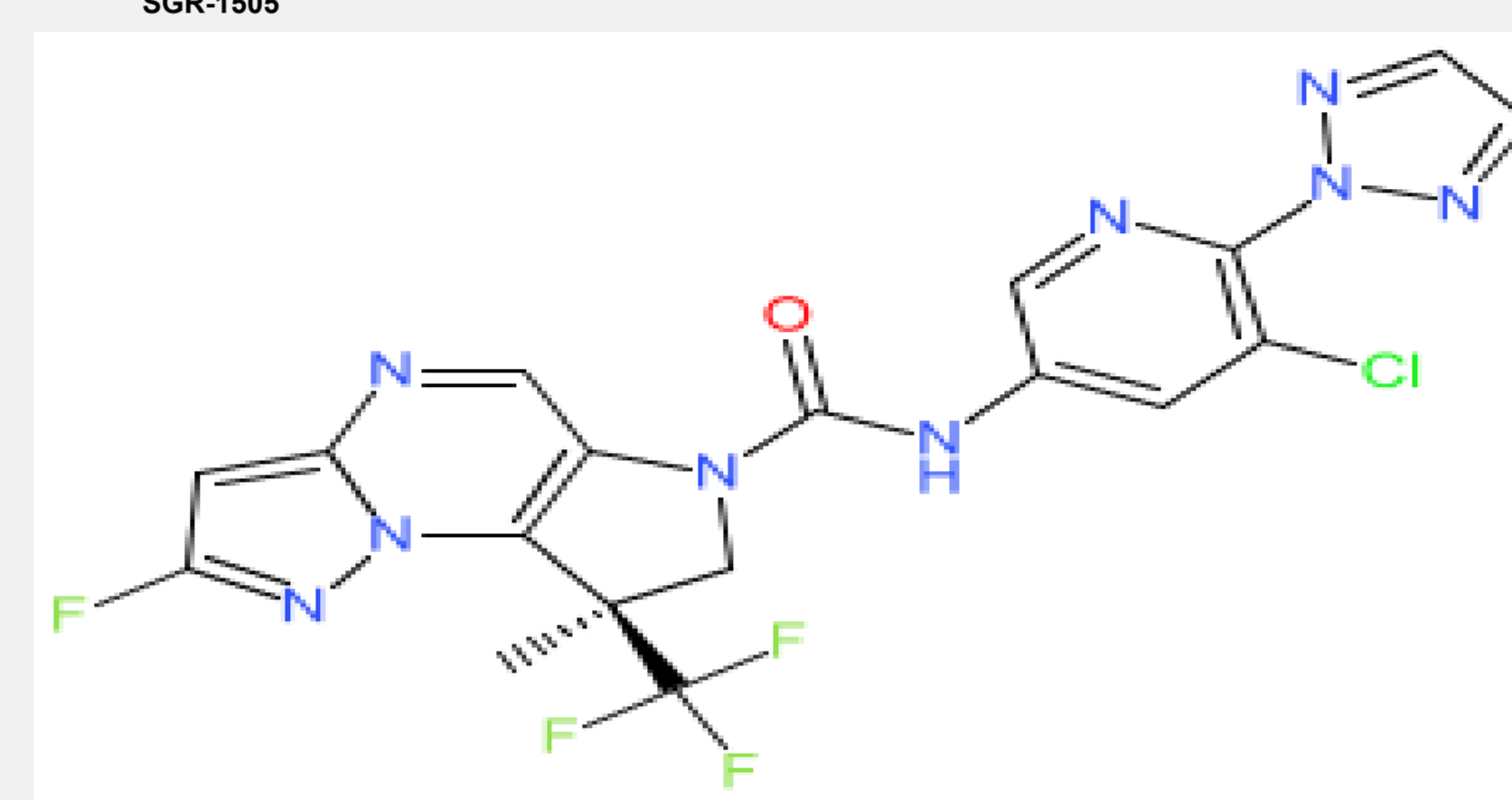
LC Conditions		
LC instrumentation (may be substituted with equivalent)	LEAP (LEAP Technologies, Chapel Hill, NC) Shimadzu SCL-30A controller with LC3010/DAAD pump (Shimadzu, Columbia, MO)	
Analytical column	Waters Xbridge C8, 2.1 x 50 mm, 5.0 μm particle size, part # 186003011	
Column temperature setting	40°C	
Autosampler temperature setting	Room temperature	
Mobile phase A	0.1% FA in water	
Mobile phase B	0.1% FA in MeCN	
Back pressure (typical)	40 bar	
Injection volume*	10.0 μL	
Needle Wash 1	2% FA in [(MeOH)/MeCN (50/50 v/v)]	
Needle Wash 2/Weak Wash	Water/MeOH (50/50 v/v)	
Flow rate	0.300 mL/min	
LC Program		
Time (minutes)	min, %B, event	
0.01	55	
0.50	55	
1.50	75	
2.00	75	
2.10	55	
2.60	Stop	

RESULTS

Accuracy and Precision of Quality Control Sample Data for SGR-1505 in Human Plasma

LLOQ QC	LQC	LMQC	MQC	HQC	
10.0 ng/mL	30.0 ng/mL	300 ng/mL	4000 ng/mL	8000 ng/mL	
9.05	29.1	283	3780	7890	
10.7	31.8	311	4150	8090	
10.7	30.3	306	4120	8270	
9.16	30.3	310	4140	8380	
10.6	31.6	279	4120	8150	
9.19	30.3	320	4080	8050	
Intraran Mean	9.9	30.6	302	4070	8140
Intraran SD	0.842	0.995	16.6	142	172
Intraran %CV	8.5	3.3	5.5	3.5	2.1
Intraran %Bias	-1	2	0.7	1.8	1.8
n	6	6	6	6	6
9.01	29.4	286	3790	7620	
10	31.2	310	4130	8260	
9.55	33.4	322	4260	8460	
10.7	34.9	313	4320	8300	
11.5	33.1	322	4210	8310	
10.9	34.3	314	4220	8230	
Intraran Mean	10.3	33.1	311	4160	8200
Intraran SD	0.924	1.92	13.3	189	293
Intraran %CV	9	5.8	4.3	4.5	3.6
Intraran %Bias	3	10.3	3.7	4	2.5
n	6	6	6	6	6
9.16	30.6	284	3550	7590	
10.7	30.6	303	3810	8750	
9.44	33.1	326	4090	8250	
9.71	30.9	340	4160	8530	
11.1	30	325	4180	8130	
9.69	32.7	314	4200	8440	
Intraran Mean	9.97	31.3	315	4000	8260
Intraran SD	0.761	1.27	19.8	263	396
Intraran %CV	7.6	4.1	6.3	6.6	4.8
Intraran %Bias	-0.3	4.3	5	0	3.3
n	6	6	6	6	6

Structure of SGR-1505

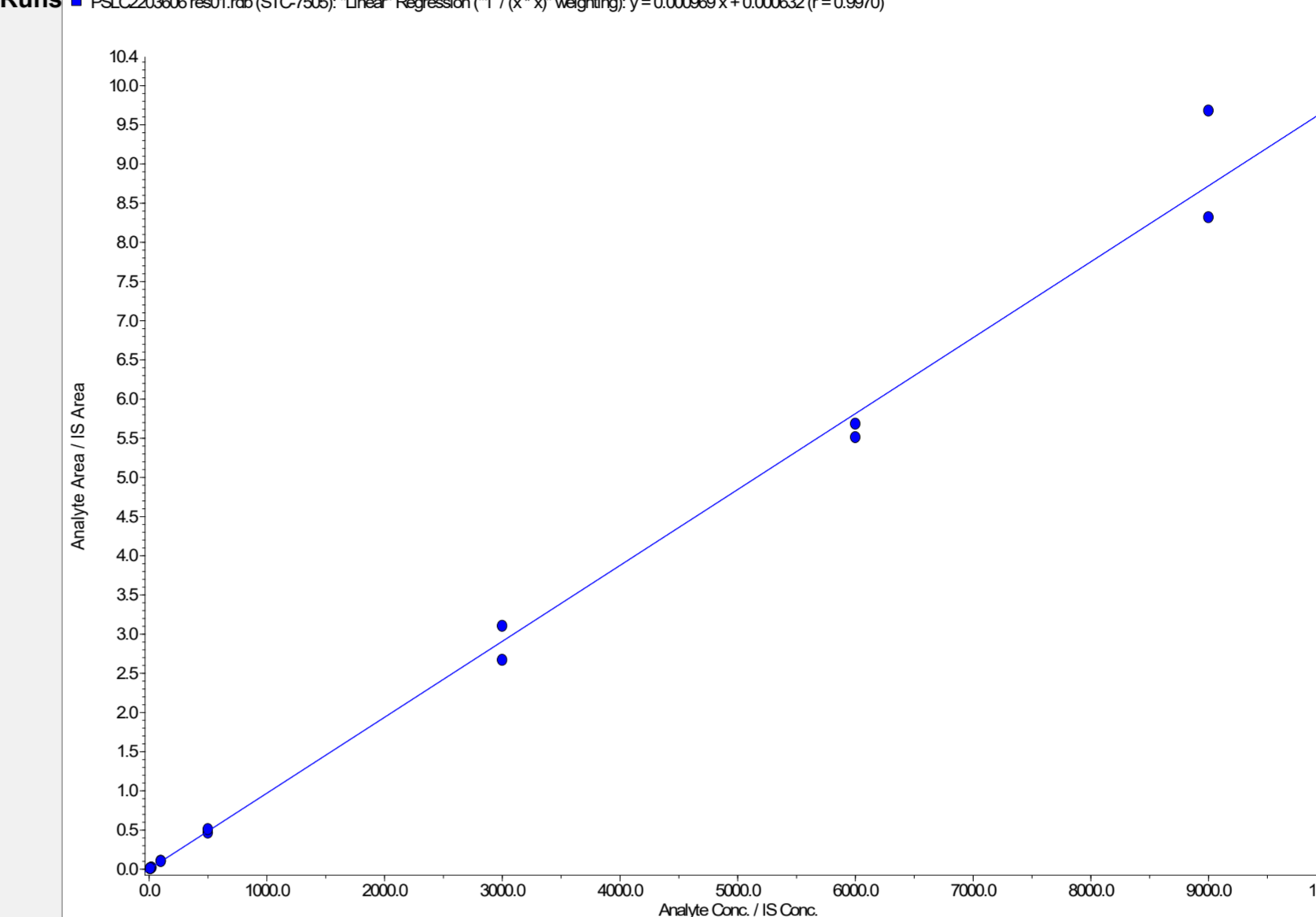


Additional Quality Control Sample Data for STC-7505 in Human Plasma

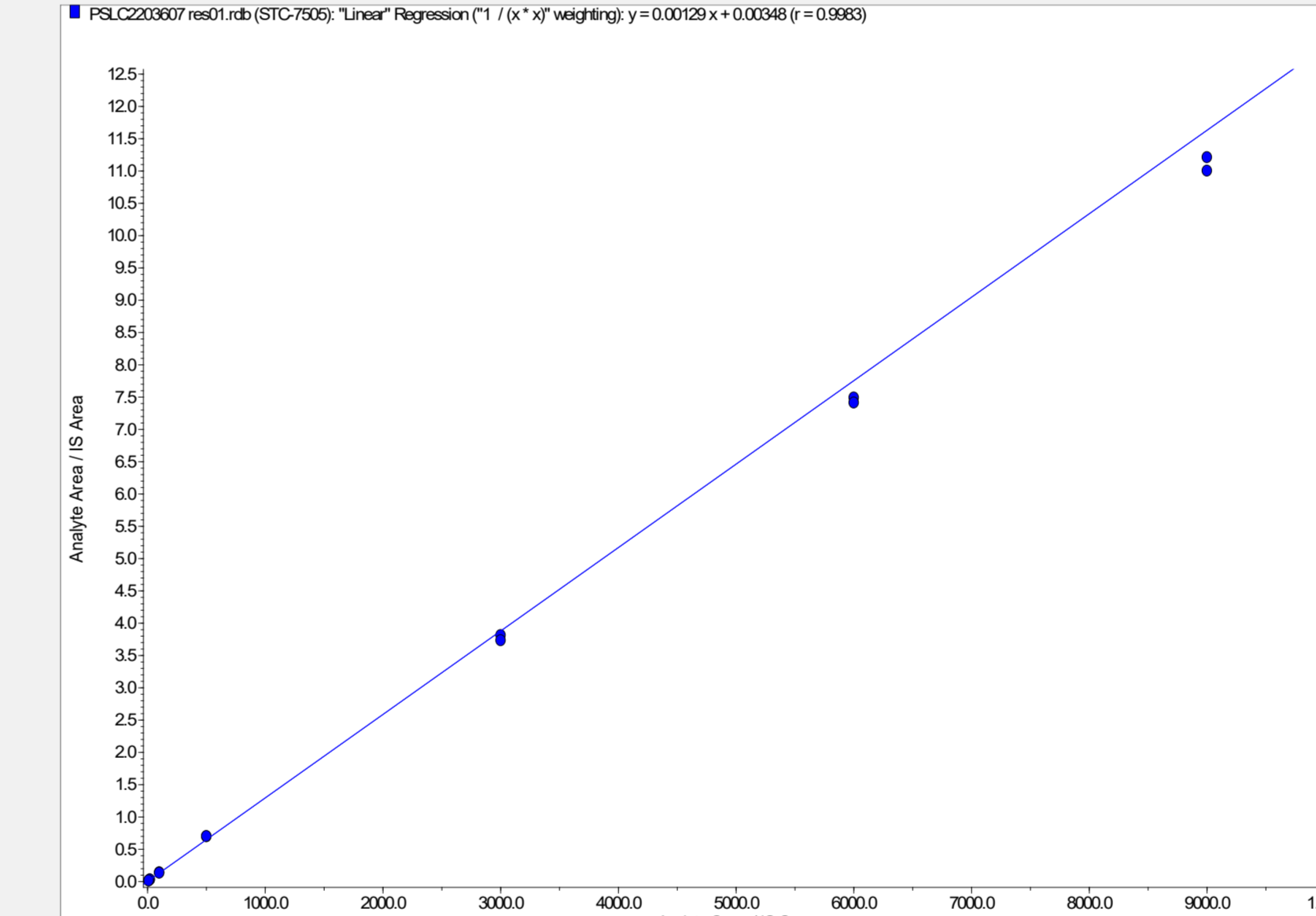
Run Date	Run Number	QC	MQC	HQC
20-Sep-2022	1	93500	4370	8030
		103000	4350	7500
		90200	3810	8200
		100000	4220	8440
		92000	3930	8360
		97100	3820	89760
Mean		96000	4080	8380
S.D.		4940	261	753
%CV		5.1	6.4	9.0
%Theoretical		96.0	102.0	104.8
%Bias		-4.0	2.0	4.8
n		6	6	6

Run Date	Run Number	Slope	Intercept	R-Squared	LLOQ	ULOQ
20-Sep-2022	1	0.00113	0.00211	0.9926	10.0	10000
22-Sep-2022	2	0.00104	0.00128	0.9969	10.0	10000
26-Sep-2022	4†	0.00104	0.000735	0.9921	10.0	10000
27-Sep-2022	5	0.00107	0.00201	0.9965	10.0	10000

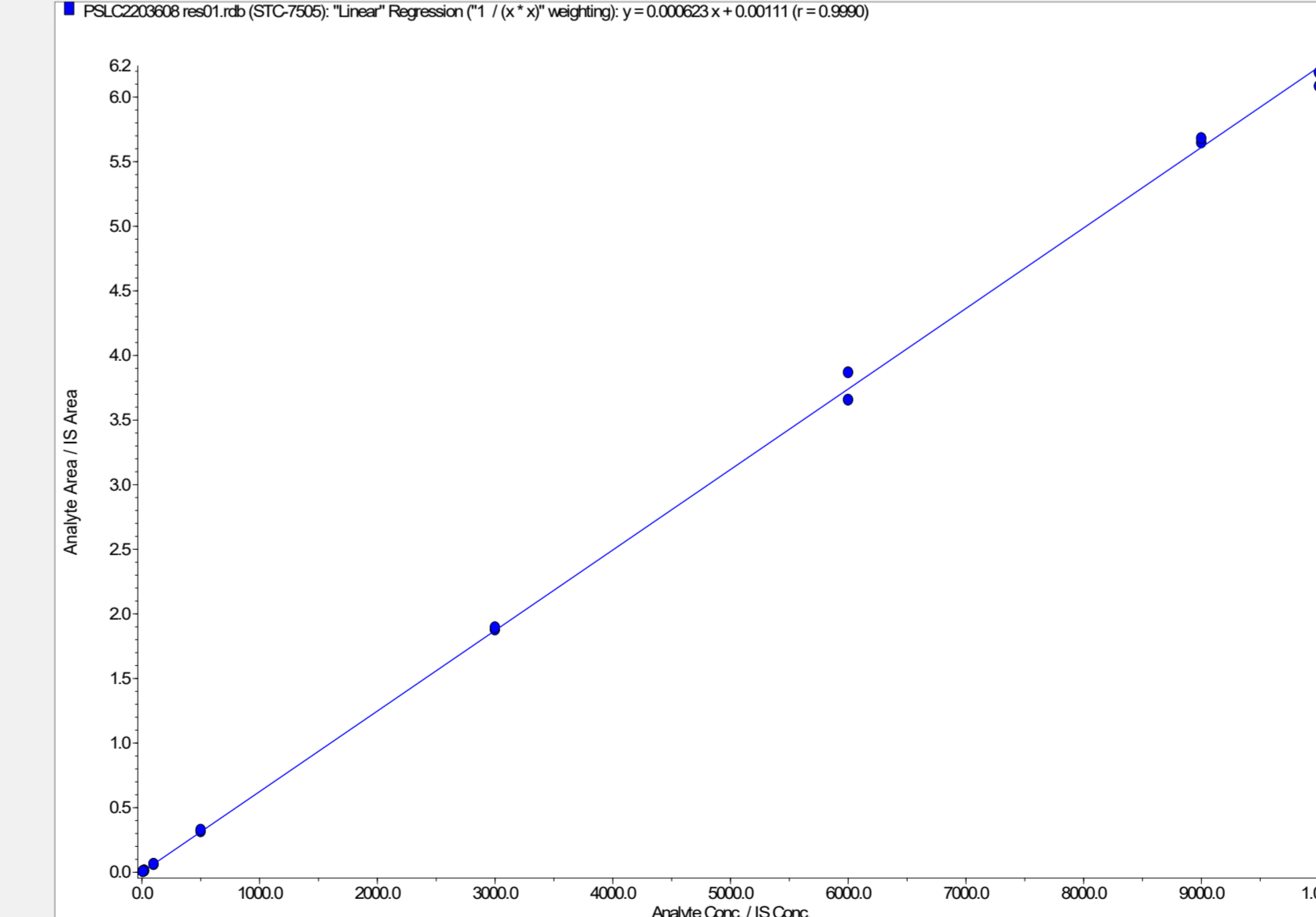
Representative Calibration Curve (Run 6)



Representative Calibration Curve (Run 7)



Representative Calibration Curve (Run 8)



Frozen Matrix Stability: SGR-1505 in Human Plasma

LQC	HQC	
30.0 ng/mL	8000 ng/mL	
30.5	7670	
30.4	7560	
30.2	7790	
31.3	8060	
31.4	7620	
32.8	7580	
Intraran Mean	31.1	7710
Intraran SD	0.967	189
Intraran %CV	3.1	2.5
Intraran %Bias	3.7	-3.6
n	6	6

Dilution Integrity (100,000 ng/mL DF=100)

QC	LQC
100000 ng/mL DF=100	30.0 ng/mL
93500	28.2
103000	29.6
90200	28.7
100000	32.1
92000	27.0
97100	27.0
Mean	28.8
S.D.	1.92
%CV	6.7
%Theoretical	96.0
%Bias	-4.0
n	6

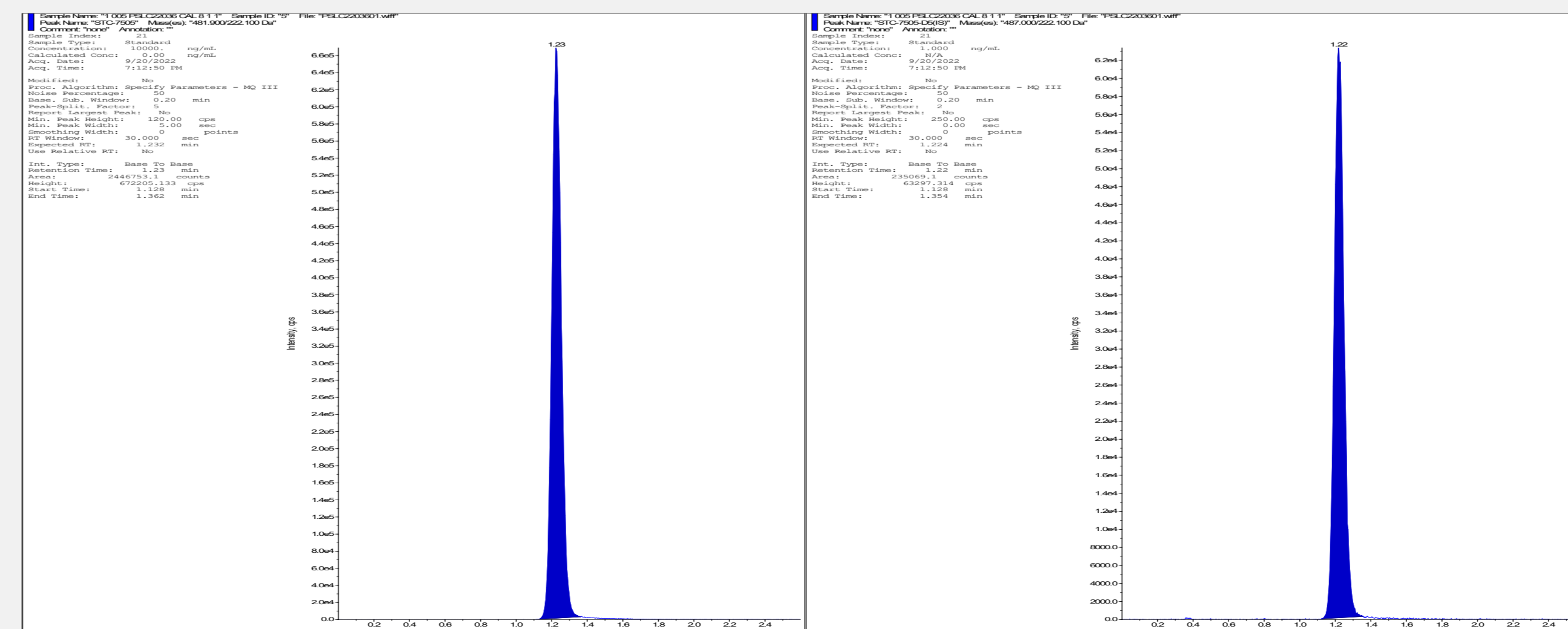
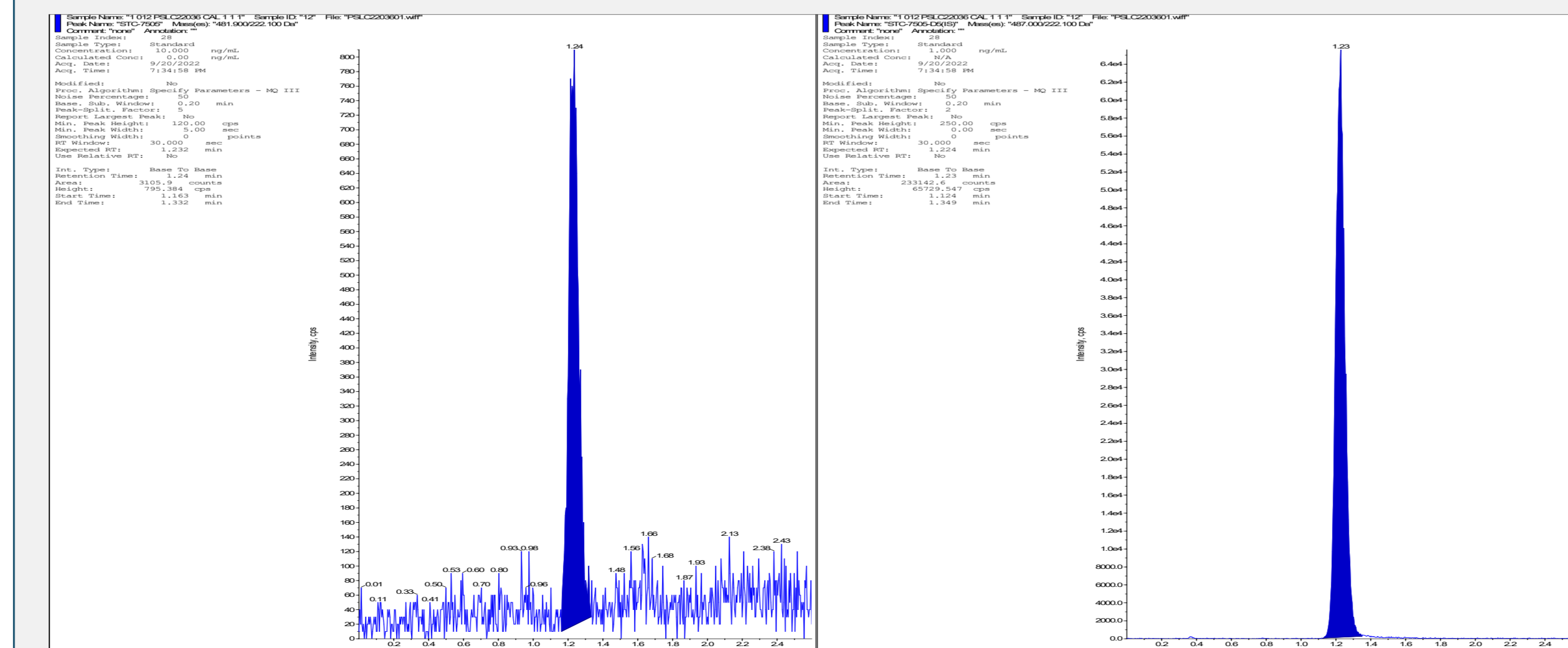
Co-Medication Interference

QC	LQC
100000 ng/mL DF=100	30.0 ng/mL
93500	28.2
103000	29.6
90200	28.7
100000	32.1
92000	27.0
97100	27.0
Mean	28.8
S.D.	1.92
%CV	6.7
%Theoretical	96.0
%Bias	-4.0
n	6

Extraction Recovery Data for SGR-1505

Low Extracted Peak Area	Low Recovery Peak Area	
5249	3545.9	
5865.3	3343.6	
6032.1	3375.7	
5493		
5465.9		
5545.1		
Mean*	5608.4	3421.7
S.D.**	287	109
%CV*	5.1	3.2
n	6	3
Low Analyte Recovery (%)	163.9	
Medium Extracted Peak Area	Medium Recovery Peak Area	
520721	417167	
576387	425249	
562666		
546704		
555458		
571367		
Mean*	555884	420858
S.D.**	20000	4090
%CV*	3.6	1
n	6	3
Medium Analyte Recovery (%)	132.1	
992795	893148	
1087583	840877	
1093268	822418	
1075680		
1078621		
988837		
Mean*	1052797	852148
S.D.**	48400	36700
%CV*	4.6	4.3
n	6	3
High Analyte Recovery (%)	123.5	

Representative Chromatogram of LLOQ (10.0 ng/mL)



CONCLUSION

The LC-MS/MS method for quantifying SGR-1505 in human K₂EDTA plasma fully achieves the study objective of delivering a reliable, high-throughput bioanalytical assay to support clinical pharmacokinetic studies of this potent MALT1 inhibitor, an emerging therapeutic target in B-cell malignancies and autoimmune diseases. Validated per ICH M10 (2022) guidelines, the method demonstrated excellent accuracy (bias ≤±9.2%) and precision (CV ≤12.4% intra-run, ≤8.4% inter-run) across five runs, meeting all criteria (≤±15%; ≤±20% at LLOQ of 10.0 ng/mL). Selectivity was confirmed in six individual plasma lots with no interference, IS-normalized matrix factor CV was ≤4.1%, and recovery was consistent at ~97% (CV <15%). Carryover was negligible, and dilution integrity was verified up to 500-fold.

Stability was robust under all ICH M10 conditions: 24 h bench-top, 4 freeze-thaw cycles (-20°C/-70°C), 234 days frozen at -20°C, 144 h processed extract, and 2 h in whole blood (RT/wet ice), with no impact from hemolyzed, hyperlipidemic, or co-medicated (20,000 ng/mL) plasma. Minor initial deviations in recovery and freeze-thaw assessments were resolved without compromising data integrity.

This sensitive (10.0–10,000 ng/mL), low-volume (20 μL) method fully complies with ICH M10, enabling accurate and efficient PK evaluation of SGR-1505 in clinical trials. By bridging discovery and clinical development through rigorous CRO-led bioanalysis, this work supports the advancement of MALT1-targeted therapies for oncology and immunology.

