

In Vitro AAV2-hRPE65 Potency Assay

3 STEPS:

- 1) Transduction of HEK293/LRAT cells with SPK-RPE65
- 2) Isomerohydrolase assay
- 3) Detection/quantification of 11-cis-retinol by LC-MS/MS

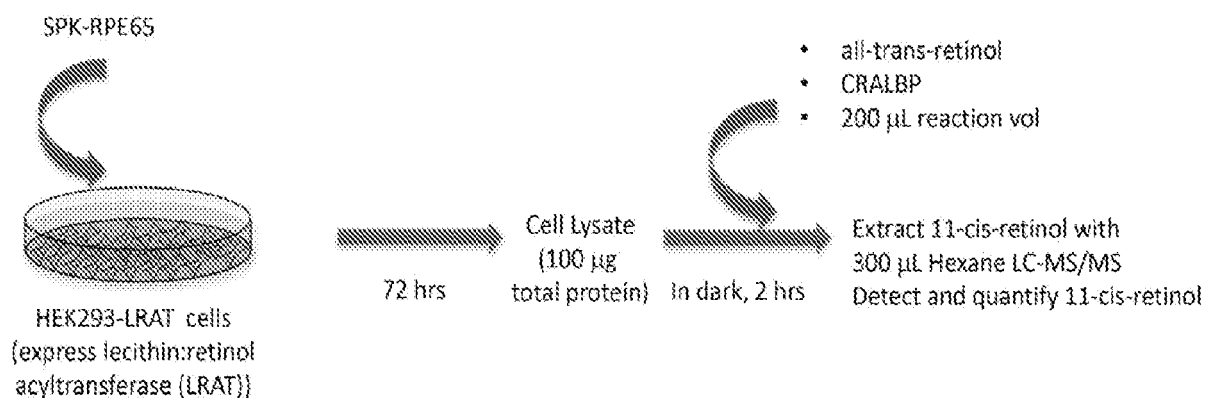


Figure 3

LC-MS/MS of retinols

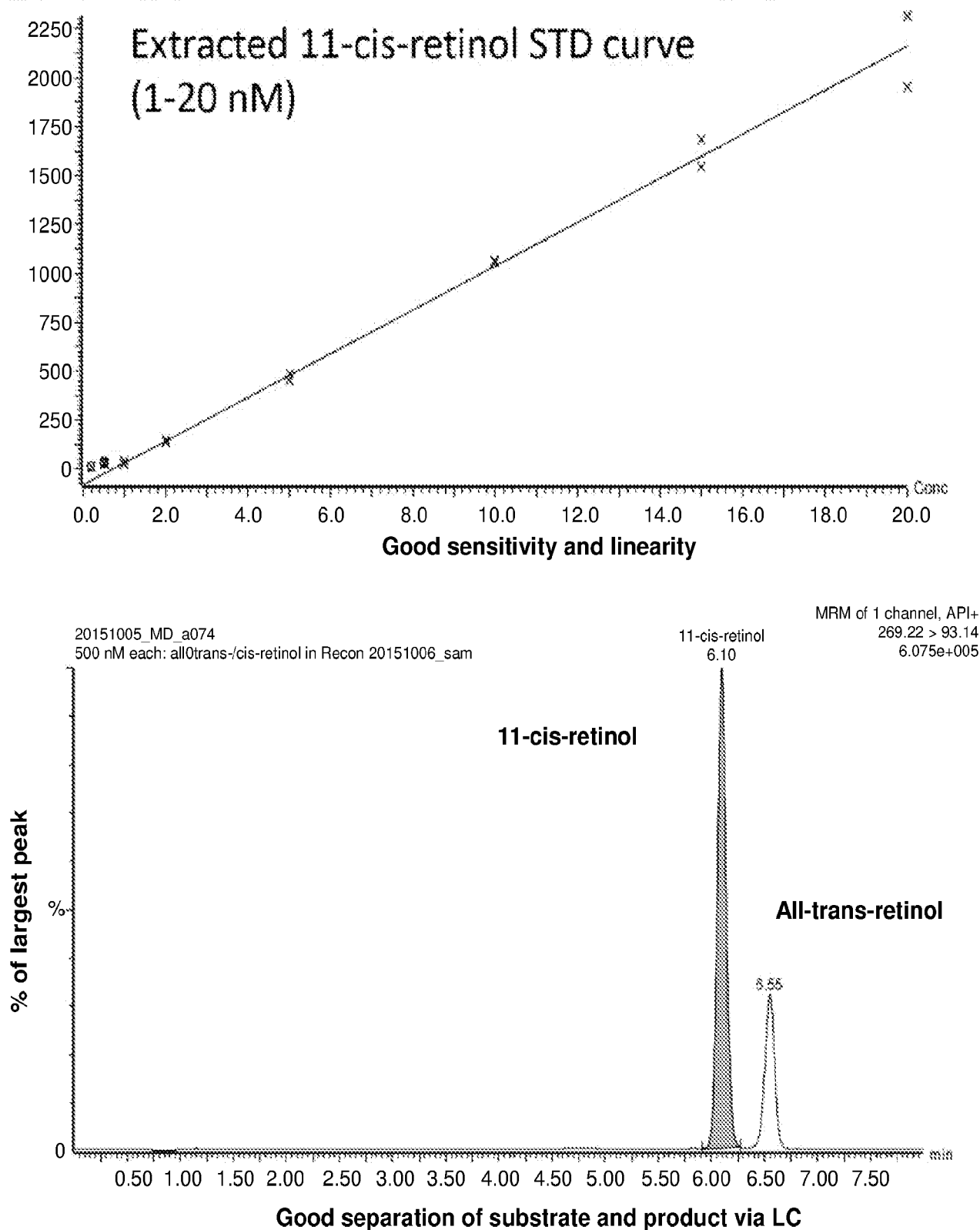
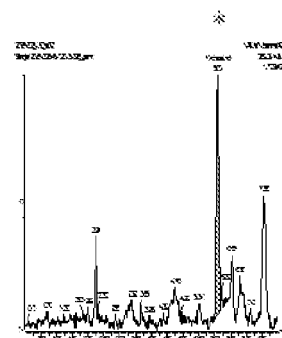
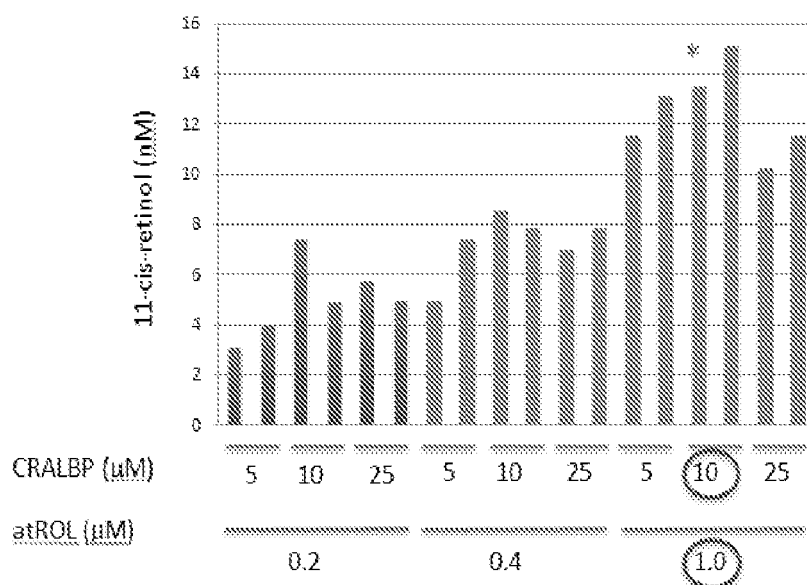


Figure 4

Assay Optimization: enzymatic reagent concentrations – CRALBP and atROL

Assay Design:

- * HEK293/LRAT cells transduced with AAV-RPE65 (1×10^5 vector genomes/cell)
- * Evaluate enzymatic assay by varying substrate and CRALBP reagents



Optimized conditions:

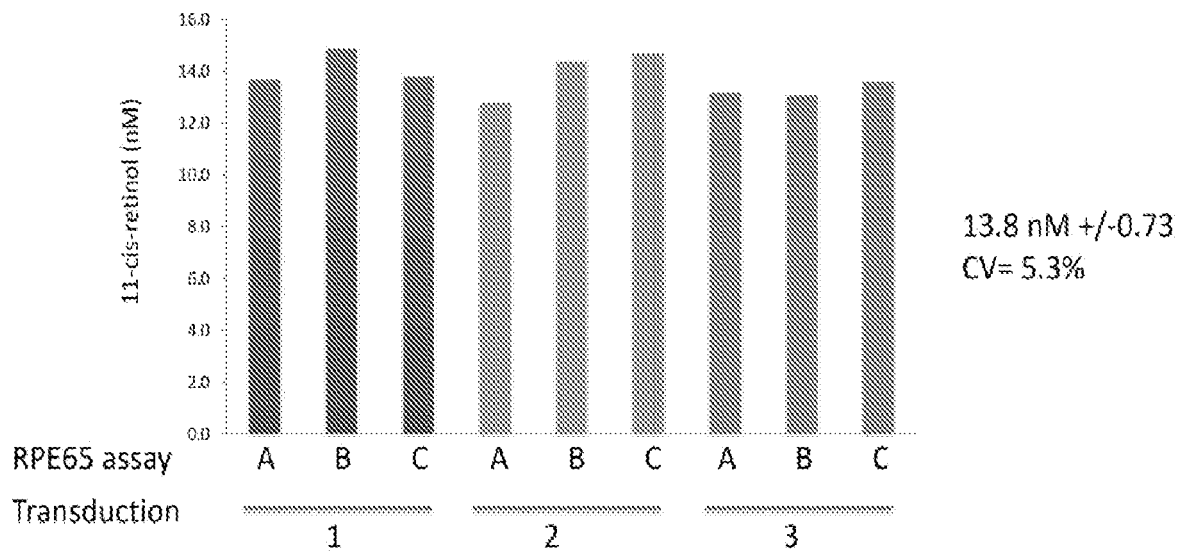
100 μg cell protein
1 μM all-trans-retinol
10 μM CRALBP

Figure 5

Reproducible transduction and enzymatic assay at single MOI

Assay Design:

- * HEK293/LRAT cells transduced with AAV-RPE65 (1×10^5 vector genomes/cell)
- * Three independent transductions (9 total assays)
- * Cell lysates analyzed for RPE65 activity (3 assays/lysate)



Dose-dependent increase of 11cROL over 8 MOIs of AAV-RPE65

Assay Design:

- * HEK293/LRAT cells transduced with AAV-RPE65 vectors
- * 8 MOIs (3×10^3 , 1×10^4 , 3×10^4 , 1×10^5 , 2.5×10^5 , 5×10^5 , 1×10^6 , 2×10^6 vector genomes/cell)
- * Three independent transductions
- * Cell lysates analyzed for RPE65 activity (3 assays/lysate)

MOI	Ave	SD	CV
3.00×10^3	BLOQ		
1.00×10^4	BLOQ		
3.00×10^4	2.2	0.436	20.11%
1.00×10^5	4.2	0.944	22.33%
2.50×10^5	5.6	1.219	18.43%
5.00×10^5	9.0	1.218	13.47%
1.00×10^6	10.7	0.756	7.04%
2.00×10^6	9.9	1.386	14.02%

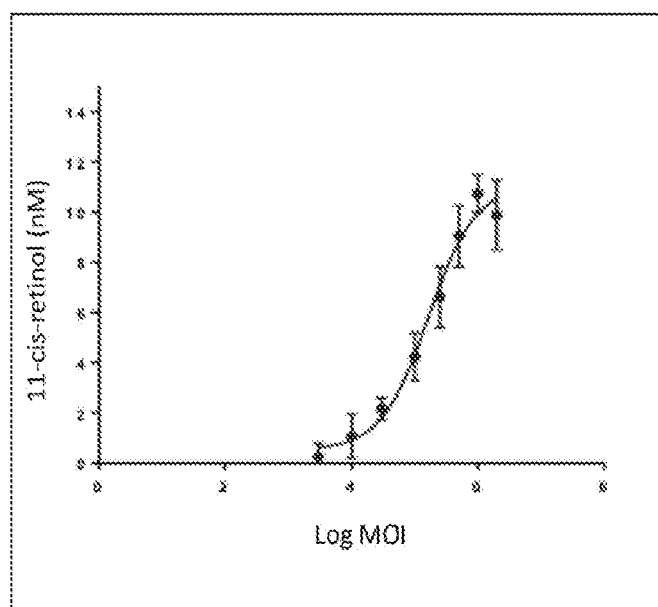
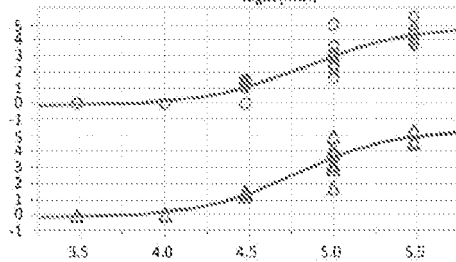
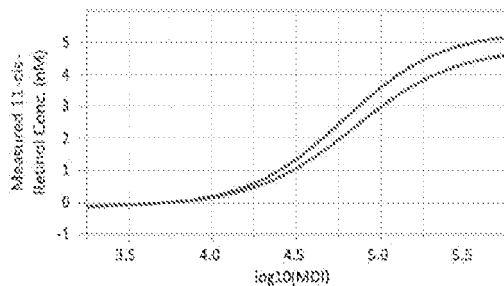


Figure 7

Use of assay to demonstrate relative potency of different lots of AAV-RPE65 at different MOIs

Assay Design:

- * HEK293/LRAT cells transduced with two different lots of AAV-RPE65
- * Five MOIs (3×10^3 , 1×10^4 , 3×10^4 , 1×10^5 , 3×10^5 vector genomes/cell)
- * Cell lysates analyzed for RPE65 activity
 - * 4 Parameter Logistic Model used to compare two lots of vector



Statistical tests for parallelism	Probability
F test	0.643
ChiSquare	0.895

Conclusion:

The Prob>F and Prob>ChiSq (p-values) are both >0.05, indicating that there are no differences between the curves.

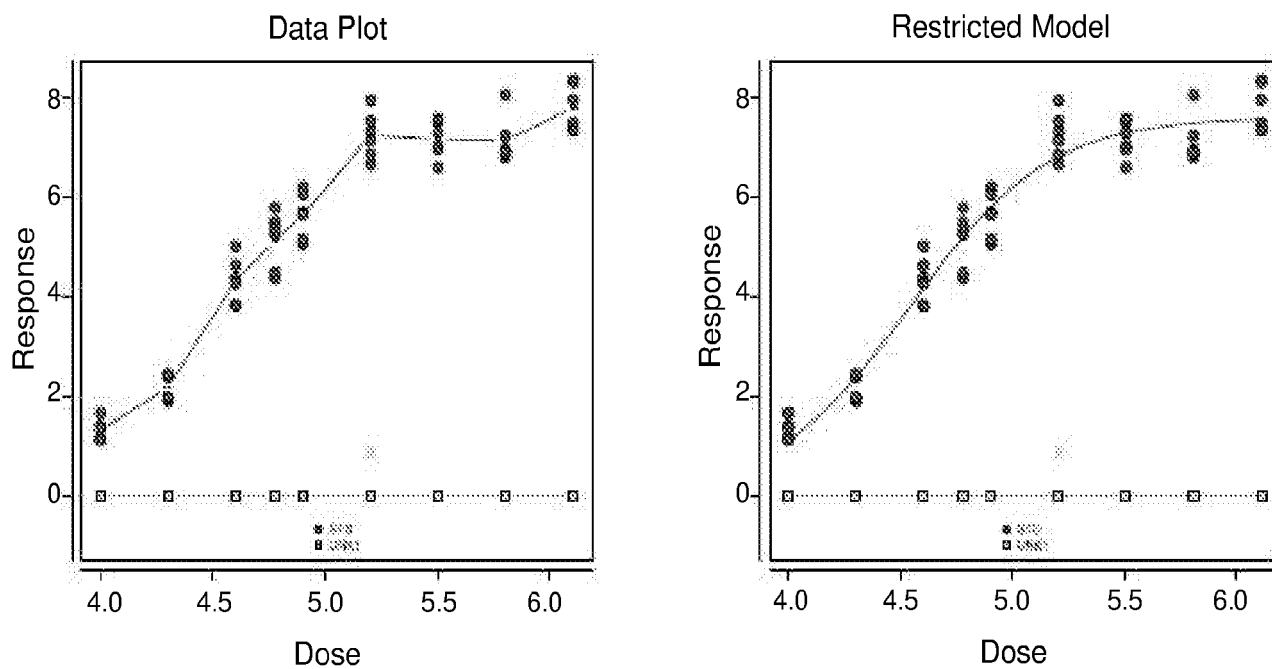
Thus, two lots of vectors are of similar potency

Figure 8

Potency Estimation

Relative Potency	UNK1	STD
Potency Ratio	6.334 E-07	
Relative Confidence Interval	0.00% - Infinity (Infinity)	

Graphics



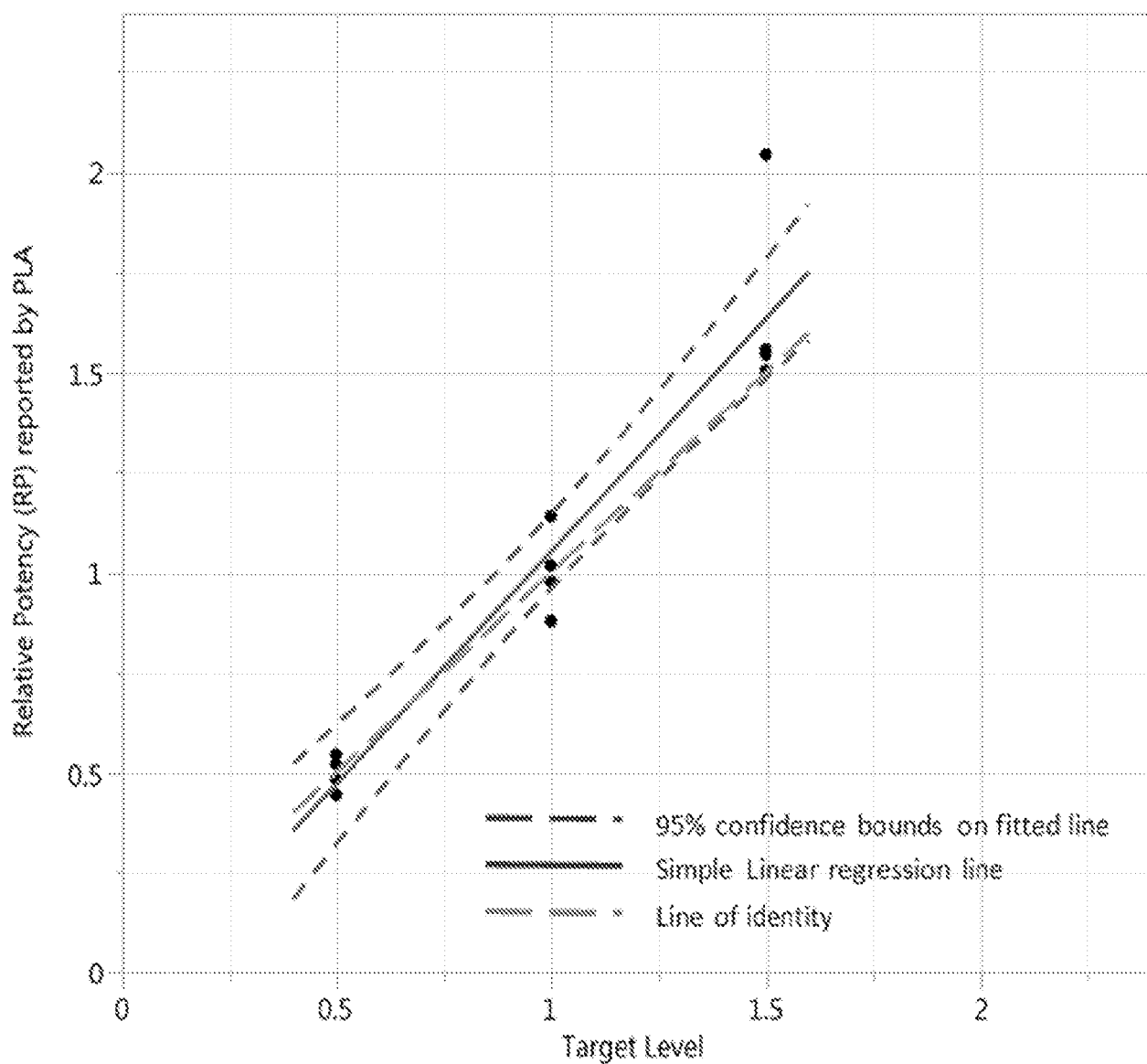


Figure 10

Level 100%								
Plate 1 Vol. Sample: _____			Plate 2 Vol. Sample: _____			Plate 3 Vol. Sample: _____		
RS MOI 1.0×10^4	RS MOI 1.0×10^4	RS MOI 1.0×10^4	RS MOI 2.0×10^4	RS MOI 2.0×10^4	RS MOI 2.0×10^4	RS MOI 4.0×10^4	RS MOI 4.0×10^4	RS MOI 4.0×10^4
TA MOI 1.0×10^4	TA MOI 1.0×10^4	TA MOI 1.0×10^4	TA MOI 2.0×10^4	TA MOI 2.0×10^4	TA MOI 2.0×10^4	TA MOI 4.0×10^4	TA MOI 4.0×10^4	TA MOI 4.0×10^4
Plate 4 Vol. Sample: _____			Plate 5 Vol. Sample: _____			Plate 6 Vol. Sample: _____		
RS MOI 6×10^4	RS MOI 6×10^4	RS MOI 6×10^4	RS MOI 8.0×10^4	RS MOI 8.0×10^4	RS MOI 8.0×10^4	RS MOI 1.6×10^5	RS MOI 1.6×10^5	RS MOI 1.6×10^5
TA MOI 6×10^4	TA MOI 6×10^4	RS MOI 6×10^4	TA MOI 8.0×10^4	TA MOI 8.0×10^4	TA MOI 8.0×10^4	TA MOI 1.6×10^5	TA MOI 1.6×10^5	TA MOI 1.6×10^5
Plate 7 Vol. Sample: _____			Plate 8 Vol. Sample: _____			Plate 9 Vol. Sample: _____		
RS MOI 3.2×10^5	RS MOI 3.2×10^5	RS MOI 3.2×10^5	RS MOI 6.4×10^5	RS MOI 6.4×10^5	RS MOI 6.4×10^5	RS MOI 1.28×10^6	RS MOI 1.28×10^6	RS MOI 1.28×10^6
TA MOI 3.2×10^5	TA MOI 3.2×10^5	TA MOI 3.2×10^5	TA MOI 6.4×10^5	TA MOI 6.4×10^5	TA MOI 6.4×10^5	TA MOI 1.28×10^6	TA MOI 1.28×10^6	TA MOI 1.28×10^6
Plate 10 Vol. Form Buf: _____								
Blank Matrix Control	Blank Matrix Control	Blank Matrix Control						
No Transducti on Control	No Transducti on Control	Cell Count Well						

Figure 11

Level 50%								
Plate 1 Vol. Sample:			Plate 2 Vol. Sample:			Plate 3 Vol. Sample:		
RS MOI 1.0×10^4	RS MOI 1.0×10^4	RS MOI 1.0×10^4	RS MOI 2.0×10^4	RS MOI 2.0×10^4	RS MOI 2.0×10^4	RS MOI 4.0×10^4	RS MOI 4.0×10^4	RS MOI 4.0×10^4
TA MOI 5.0×10^3	TA MOI 5.0×10^3	TA MOI 5.0×10^3	TA MOI 1.0×10^4	TA MOI 1.0×10^4	TA MOI 1.0×10^4	TA MOI 2.0×10^4	TA MOI 2.0×10^4	TA MOI 2.0×10^4
Plate 4 Vol. Sample:			Plate 5 Vol. Sample:			Plate 6 Vol. Sample:		
RS MOI 6×10^4	RS MOI 6×10^4	RS MOI 6×10^4	RS MOI 8.0×10^4	RS MOI 8.0×10^4	RS MOI 8.0×10^4	RS MOI 1.6×10^5	RS MOI 1.6×10^5	RS MOI 1.6×10^5
TA MOI 3.0×10^4	TA MOI 3.0×10^4	TA MOI 3.0×10^4	TA MOI 4.0×10^4	TA MOI 4.0×10^4	TA MOI 4.0×10^4	TA MOI 8.0×10^4	TA MOI 8.0×10^4	TA MOI 8.0×10^4
Plate 7 Vol. Sample:			Plate 8 Vol. Sample:			Plate 9 Vol. Sample:		
RS MOI 3.2×10^5	RS MOI 3.2×10^5	RS MOI 3.2×10^5	RS MOI 6.4×10^5	RS MOI 6.4×10^5	RS MOI 6.4×10^5	RS MOI 1.28×10^6	RS MOI 1.28×10^6	RS MOI 1.28×10^6
TA MOI 1.6×10^5	TA MOI 1.6×10^5	TA MOI 1.6×10^5	TA MOI 3.2×10^5	TA MOI 3.2×10^5	TA MOI 3.2×10^5	TA MOI 6.4×10^5	TA MOI 6.4×10^5	TA MOI 6.4×10^5
Plate 10 Vol. Form Buf:								
Blank Matrix Control	Blank Matrix Control	Blank Matrix Control						
No Transducti on Control	No Transducti on Control	Cell Count Well						

Figure 12

Level 150%								
Plate 1 Vol. Sample: _____			Plate 2 Vol. Sample: _____			Plate 3 Vol. Sample: _____		
RS MOI 1.0×10^4	RS MOI 1.0×10^4	RS MOI 1.0×10^4	RS MOI 2.0×10^4	RS MOI 2.0×10^4	RS MOI 2.0×10^4	RS MOI 4.0×10^4	RS MOI 4.0×10^4	RS MOI 4.0×10^4
TA MOI 1.5×10^4	TA MOI 1.5×10^4	TA MOI 1.5×10^4	TA MOI 3.0×10^4	TA MOI 3.0×10^4	TA MOI 3.0×10^4	TA MOI 6.0×10^4	TA MOI 6.0×10^4	TA MOI 6.0×10^4
Plate 4 Vol. Sample: _____			Plate 5 Vol. Sample: _____			Plate 6 Vol. Sample: _____		
RS MOI 6×10^4	RS MOI 6×10^4	RS MOI 6×10^4	RS MOI 8.0×10^4	RS MOI 8.0×10^4	RS MOI 8.0×10^4	RS MOI 1.6×10^5	RS MOI 1.6×10^5	RS MOI 1.6×10^5
TA MOI 9.0×10^4	TA MOI 9.0×10^4	TA MOI 9.0×10^4	TA MOI 1.2×10^5	TA MOI 1.2×10^5	TA MOI 1.2×10^5	TA MOI 2.4×10^5	TA MOI 2.4×10^5	TA MOI 2.4×10^5
Plate 7 Vol. Sample: _____			Plate 8 Vol. Sample: _____			Plate 9 Vol. Sample: _____		
RS MOI 3.2×10^5	RS MOI 3.2×10^5	RS MOI 3.2×10^5	RS MOI 6.4×10^5	RS MOI 6.4×10^5	RS MOI 6.4×10^5	RS MOI 1.28×10^6	RS MOI 1.28×10^6	RS MOI 1.28×10^6
TA MOI 4.8×10^5	TA MOI 4.8×10^5	TA MOI 4.8×10^5	TA MOI 9.6×10^5	TA MOI 9.6×10^5	TA MOI 9.6×10^5	TA MOI 1.92×10^6	TA MOI 1.92×10^6	TA MOI 1.92×10^6
Plate 10 Vol. Form Buf: _____								
Blank Matrix Control	Blank Matrix Control	Blank Matrix Control						
No Transducti on Control	No Transducti on Control	Cell Count Well						

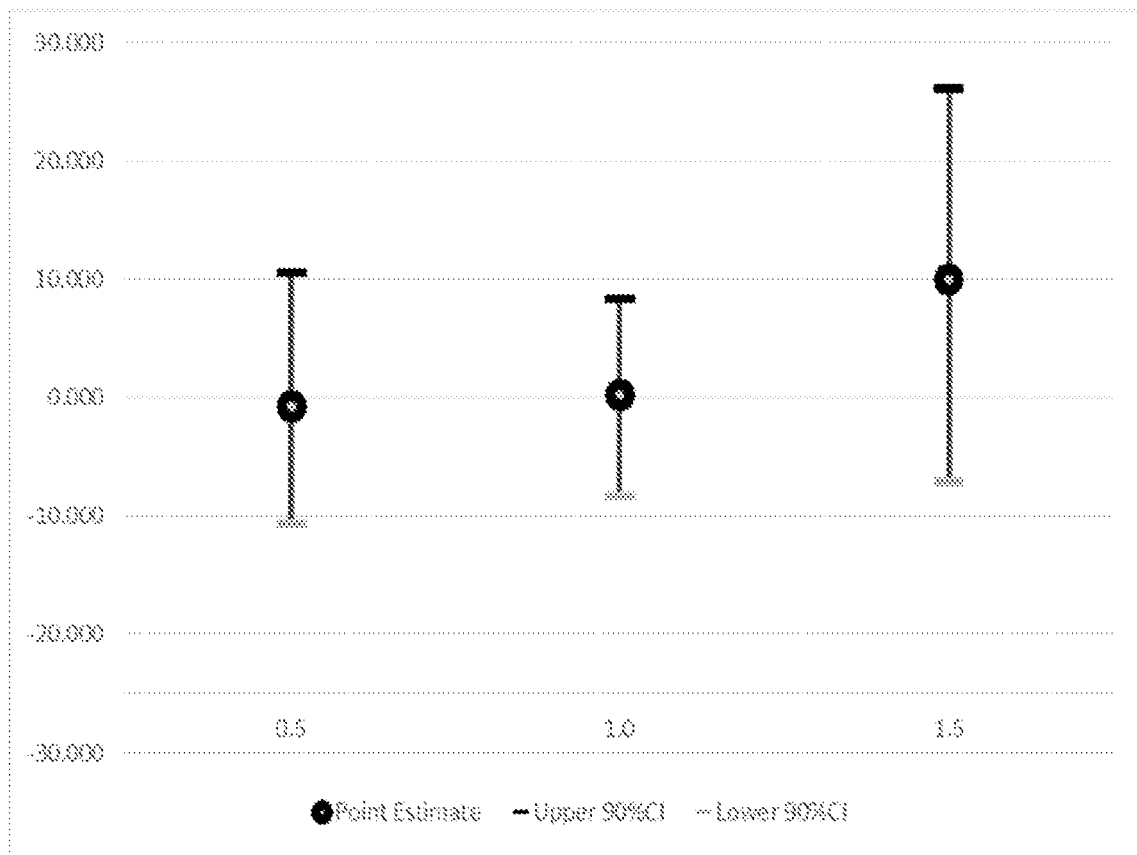
Figure 13

Figure 14

Potency Estimation

Relative Potency	UNK1	STD
Potency Ratio	0.46255	
90% Confidence Interval	0.43621 - 0.53137	
Relative Confidence Interval	90.61% - 110.12% (19.31%)	

Graphics

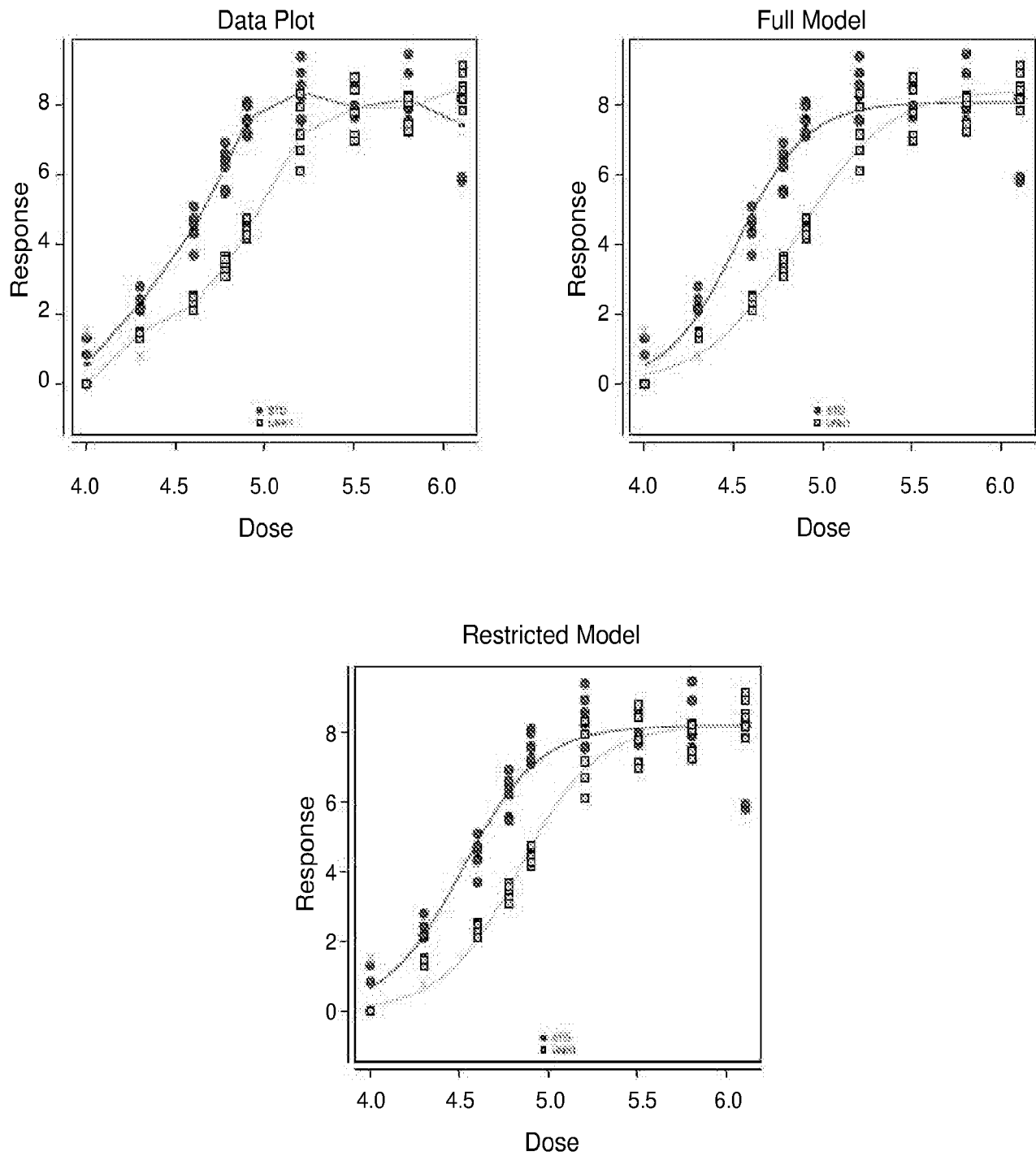


Figure 15

Potency Estimation

Relative Potency	UNK1	STD
Potency Ratio	0.44382	
90% Confidence Interval	0.38764 - 0.50769	
Relative Confidence Interval	67.39% - 114.44% (27.05%)	

Graphics

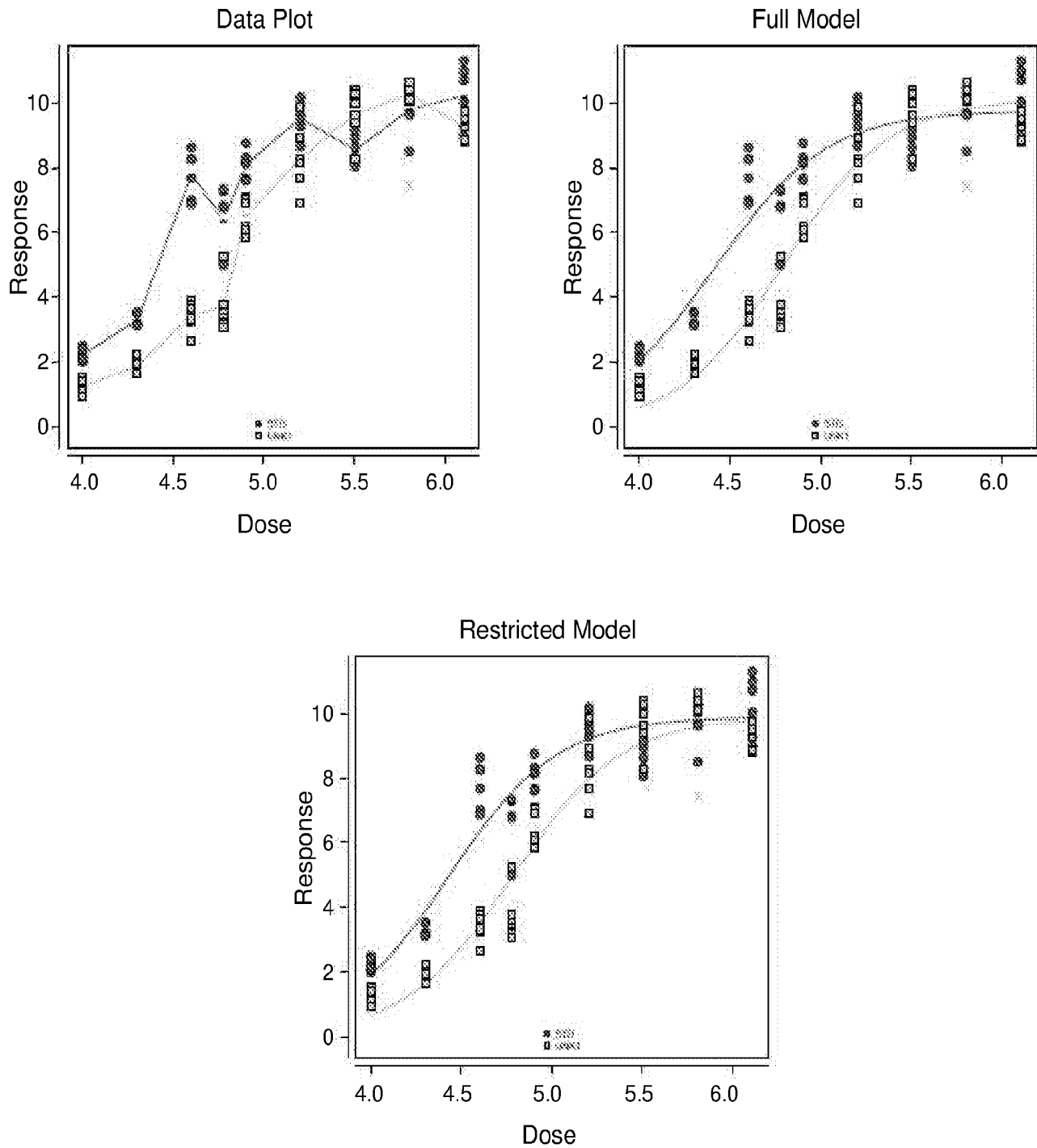


Figure 16**Potency Estimation**

Relative Potency	UNK	STD
Potency Ratio	0.97526	
90% Confidence Interval	0.86501 - 1.07591	
Relative Confidence Interval	90.65% - 110.31% (19.66%)	

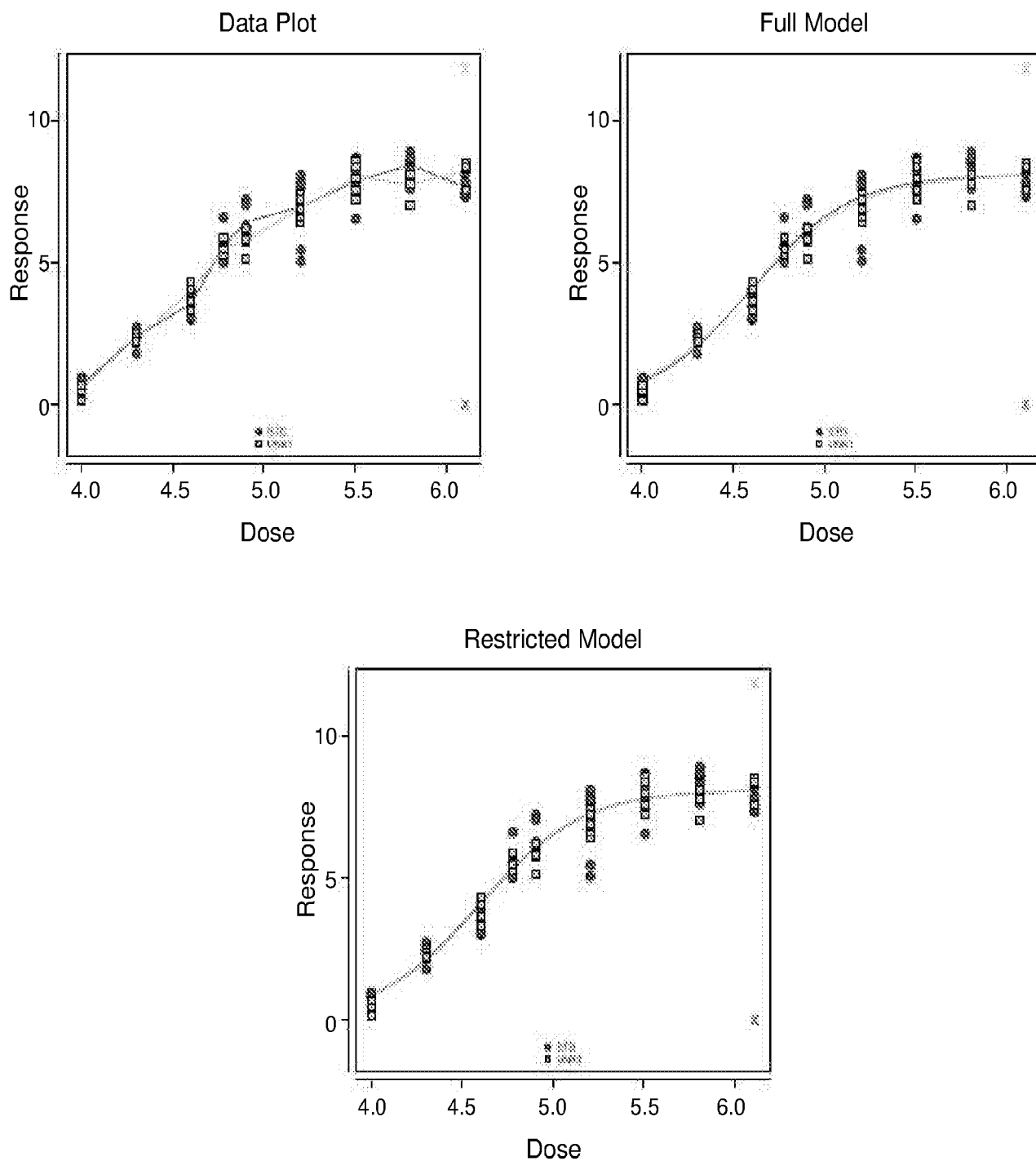
Graphics

Figure 17

Potency Estimation

Relative Potency	UNK1	STD
Potency Ratio	1.01627	
90% Confidence Interval	0.86813 - 1.16970	
Relative Confidence Interval	85.42% - 117.07% (31.54%)	

Graphics

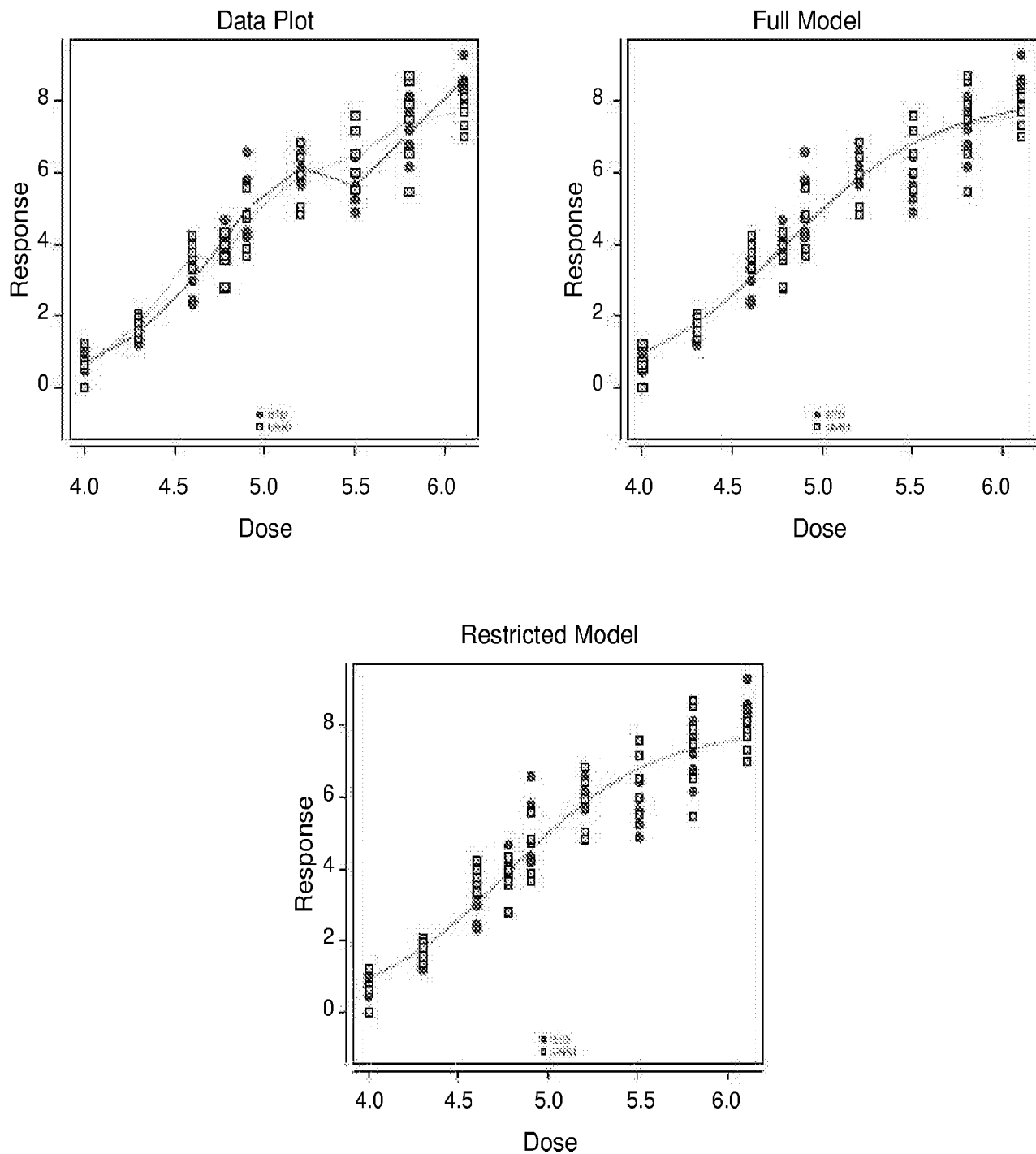


Figure 18

Potency Estimation

Relative Potency	UNK1	STD
Potency Ratio	1.50430	
90% Confidence Interval	1.34530 - 1.68343	
Relative Confidence Interval	89.39% - 111.85% (22.47%)	

Graphics

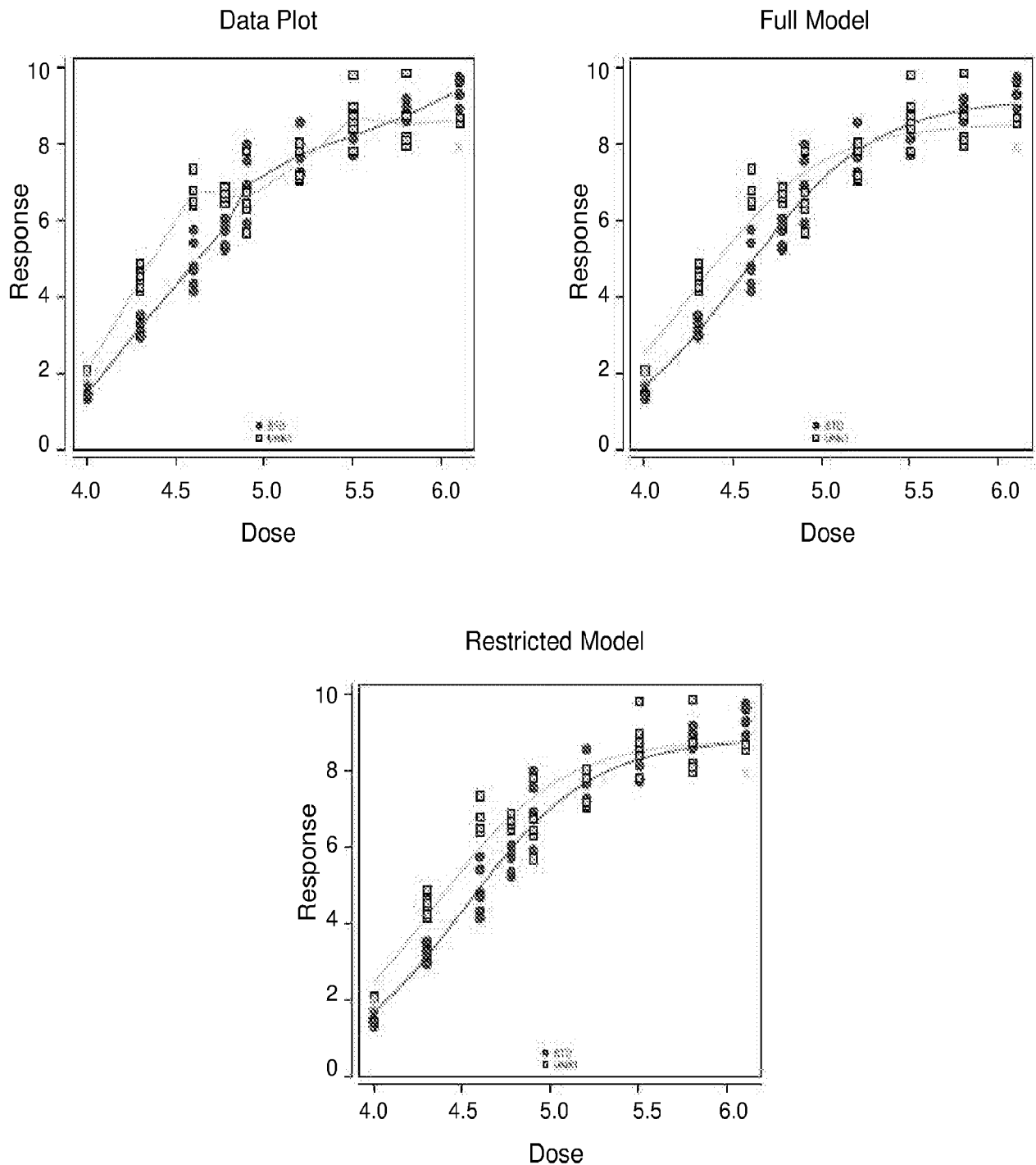


Figure 19

Potency Estimation

Relative Potency	LNK1	STD
Potency Ratio	1.55749	
90% Confidence Interval	1.29817 - 1.86881	
Relative Confidence Interval	63.35% - 119.98% (35.63%)	

Graphics

