

Linearity

CLONIDINE drug product

Essay	Observed AUC (mAU)	Concentration (µg/L)	Variance s ²	Estimated AUC	Adjusted AUC	Confidence Interval
70% D1	138.46321	21	5.366200103	130.7374619	138.46321	-1.071906914
70% D2	127.2393	21		130.7374619	127.2393	-1.648031782
70% D3	131.7208	21		130.7374619	131.7208	0.462839888
80% D1	149.2143	24	1.211610708	149.2143	149.2143	-0.079885146
80% D2	149.2143	24		149.2143	149.2143	0.040173733
80% D3	151.2039	24		149.2143	151.2039	0.938666681
90% D1	170.1337	27	3.82240092	167.6913381	170.1337	1.150724235
90% D2	166.4311	27		167.6913381	166.4311	-0.998213131
90% D3	167.9397	27		167.6913381	167.9397	0.248068711
100% D1	188.9042	30	0.37068403	186.1679762	188.9042	1.380727354
100% D2	187.797	30		186.1679762	187.797	0.76145534
100% D3	188.7695	30		186.1679762	188.7695	1.23035519
110% D1	202.9627	33	7.455189213	204.6448143	202.9627	-0.792466981
110% D2	201.6461	33		204.6448143	201.6461	-2.698466035
110% D3	206.4397	33		204.6448143	206.4397	0.844959139
120% D1	222.2938	36	2.185793545	223.116524	222.2938	-0.446789836
120% D2	221.1741	36		223.116524	221.1741	-0.441705143
120% D3	224.9177	36		223.116524	224.9177	0.844342328
130% D1	240.1133	39	6.705046103	241.5984905	240.1133	-0.695121443
130% D2	238.8034	39		241.5984905	238.8034	-1.316805243
130% D3	243.8024	39		241.5984905	243.8024	1.638291834

Linear regression (LINEST function)			
Slope b	6.15894632	1.39959238	Intercept a
S slope	0.077139913	2.361844766	S intercept
R ²	0.99702374	2.121680218	S inv
F	6364.817361	19	DOF
Regression sum of squares	28077.05786	85.6056142	Residual sum of squares

CHECKING THE FIT TO THE LINEAR MODEL n=3 k=7			
Cochran's C test: Study of the homogeneity of a series of variances.			
C test	0.27925886	k: number of samples per test	
Cochran's critical C, α = 5%		C test = max s ² /sum s ²	0.27402886
Maximum s ²	7.455189213		
Sum s ²	27.11708719		
The variances are homogeneous at the risk of 0.05% because 0.27925886 < 0.27402886			

Calculation of experimental variance s _{exp} ²			
s _{exp} ²	3.873869599		
Calculation of midfit variance			
SCE _{all}	46.86691821		
s _{all} ²	15.62230607		
Fisher Test			
H ₀ :	Not significantly different		
H ₁ :	Significantly different		
F _{exp}	0.03739372	F threshold limit 5% (5, 14)	2.958248913
Conclusion	p-value > 5%, therefore no rejection of H ₀ . Fit to the validated linear model.	p-value	0.017783205

DEPENDENCE VERIFICATION. F(1, n - 2)			
H ₀ :	The variances are not significantly different		
H ₁ :	The variances are significantly different		
F _{exp}	6364.817361	F threshold limit 5% (1, 19)	4.38
Conclusion	Dependence is validated as H ₀ is rejected, p-value < 5%	p-value	1.80856E-25

VALIDATION OF THE PROPORTIONAL MODEL

Regression of CLONIDINE within the mixture: Comparison of the y intercept to 0		CLONIDINE Pure drug Product Regression: Comparing the intercept to 0	
df	1.39959238	df	3.803790476
Standard deviation of the inter	2.361844766	Standard deviation	2.2821159
Student Test		Student Test	
H ₀ :	The y-intercept is equal to 0	H ₀ :	The y-intercept is equal to 0
H ₁ :	The y-intercept is different from 0	H ₁ :	The y-intercept is different from 0
t _{exp}	0.59285617	t _{exp}	1.666782338
Nb of DOF (n1-n2-4)	19	Nb of DOF (n-2)	19
t threshold (5%, student)	2.093	t threshold (5%, student)	2.093
Conclusion	temp < t threshold → No rejection of H ₀ at 5% risk. The y-intercept is not significantly different from 0 the correlation line passes through the origin	Conclusion	temp < t threshold → No rejection of H ₀ at 5% risk. The y-intercept is not significantly different from 0 the correlation line passes through the origin

CALCULATED LIMIT OF DETECTION	3.446417801
CALCULATED LIMIT OF QUANTIFICATION	1.137317874

COMPARISON OF CLONIDINE IN THE MIXTURE AND THE PURE DRUG PRODUCT REGRESSIONS

Comparison of Student slopes			
H ₀ :	No difference between the two slopes		
H ₁ :	Difference between the two slopes		
t _{exp}	0.505706633		
Nb of DOF (n1-n2-4)	38		
t threshold (5%, student) α 38	2.042		
Conclusion	temp < t threshold → No rejection of H ₀ at 5% risk, no significant difference between the 2 slopes.		

COMPARISON OF STUDENT INTERCEPTS			
H ₀ :	No difference between the two intercepts		
H ₁ :	Difference between the two intercepts		
F _{exp}	0.730059602		
Nb of DOF (n1-n2-4)	38		
F threshold (5%, student) α 38	2.042		
Conclusion	temp < t threshold → No rejection of H ₀ at 5% risk therefore no systematic error.		

SIGNIFICANCE TEST OF CLONIDINE IN THE MIXTURE FORM CORRELATION COEFFICIENT (Pearson correlation coefficient test)			
H ₀ :	R=0	R reconstituted form:	0.998510753
H ₁ :	R different from 0		
t _{exp}	79.77980547		
Nb of DOF	19		
t threshold (5%, student)	2.093		
Conclusion	temp > t threshold → Rejection of H ₀ at 5% risk, evidence of a significant linear link between the variables		

SIGNIFICANCE TEST OF CLONIDINE PURE PRODUCT CORRELATION COEFFICIENT (Pearson correlation coefficient test)			
H ₀ :	R=0	R pure product:	0.99863354
H ₁ :	R different from 0		
t _{exp}	83.29480025		
Nb of DOF	19		
t threshold (5%, student)	2.093		
Conclusion	temp > t threshold → Rejection of H ₀ at 5% risk, evidence of a significant linear link between the variables		

Linearity

CLONIDINE within the mixture

Essay	Observed AUC (mAU)	Concentration (µg/L)	Variance s ²	Estimated AUC	Adjusted AUC	Confidence Interval
70% D1	134.8272	21	0.46820889	134.8272	134.8272	-0.11407656
70% D2	134.3452	21		134.2816905	134.3452	0.030606506
70% D3	133.5634	21		134.2816905	133.5634	-0.360218786
80% D1	151.8987	24	1.997199603	151.8987	151.8987	-0.096613098
80% D2	152.5736	24		152.9213905	152.5736	-0.169573122
80% D3	154.6121	24		152.9213905	154.6121	0.814831393
90% D1	168.6953	27	6.78426988	171.5610905	168.6953	-1.39721036
90% D2	173.8201	27		171.5610905	173.8201	1.13451244
90% D3	170.0483	27		171.5610905	170.0483	-0.54250393
100% D1	190.2357	30	4.216844103	190.2007905	191.596	0.017029929
100% D2	191.4622	30		190.2007905	191.4622	0.61025049
100% D3	187.4468	30		190.2007905	187.4468	-1.342770415
110% D1	206.9355	33	5.324505703	208.8404905	206.9355	-0.58851241
110% D2	211.0744	33		208.8404905	211.0744	1.69191932
110% D3	210.7729	33		208.8404905	210.7729	0.94219038
120% D1	225.7488	36	0.273447588	227.74801905	225.7488	-1.16111941
120% D2	228.7038	36		227.74801905	228.7038	0.59659529
120% D3	229.1943	36		227.74801905	229.1943	0.83757991
130% D1	246.5394	39	11.14275396	246.1188905	246.5394	-0.170663918
130% D2	247.9516	39		246.1188905	247.9516	0.83091453
130% D3	241.276	39		246.1188905	241.276	-2.36174843

Linear regression (LINEST function)			
Slope b	6.212333333	3.803790476	Intercept a
S slope	0.074593262	2.2821159	S intercept
R ²	0.997388947	2.060974434	S inv
F	6938.023749	19	DOF
Regression sum of squares	29184.82695	79.9235823	Residual sum of squares

CHECKING THE FIT TO THE LINEAR MODEL n=3 k=7			
Cochran's C test: Study of the homogeneity of a series of variances.			
C test	0.36887408	k: number of samples per test	
Cochran's critical C, α = 5%	0.361	C test = max s ² /sum s ²	0.36887408
Maximum s ²	11.14275396		
Sum s ²	30.20739972		
The variances are homogeneous at the risk of 0.05% because 0.36887408 < 0.361			

Calculation of experimental variance s _{exp} ²			
s _{exp} ²	4.315342818		
Calculation of midfit variance			
SCE _{all}	36.77015412		
s _{all} ²	12.25671804		
Fisher Test			
H ₀ :	Not significantly different		
H ₁ :	Significantly different		
F _{exp}	2.84026207	F threshold limit 5% (5, 16)	2.958248913
Conclusion	p-value > 5%, therefore no rejection of H ₀ . Fit to the validated linear model!	p-value	0.05547278

DEPENDENCE VERIFICATION. F(1, n - 2)			
H ₀ :	The variances are not significantly different		
H ₁ :	The variances are significantly different		
F _{exp}	6938.023749	F threshold limit 5% (1, 19)	4.38
Conclusion	Dependence is validated as H ₀ is rejected, p-value < 5%	p-value	7.9896E-26

Precision CLONIDINE drug product

Essay Number	AUC (mAu*min)	Concentration (µg/mL)	Observed concentration (µg/mL)	Recovery Rate	
Day 1-1	178,9556	30	28,82895935	1,039034688	Average:
Day 1-2	181,677	30	29,27082066	1,024305978	103%
Day 1-3	181,3668	30	29,2204549	1,025984837	Variance:
Day 1-4	183,1679	30	29,5128913	1,016236957	7,6584E-05
Day 1-5	180,3336	30	29,05269893	1,031576702	
Day 1-6	179,1386	30	28,85867222	1,038044259	
Day 2-1	183,9416	30	29,63851344	1,012049552	Average :
Day 2-2	192,3185	30	30,99863252	0,966712249	99%
Day 2-3	190,7145	30	30,73819835	0,975393388	Variance :
Day 2-4	182,3381	30	29,37816046	1,020727985	0,000459909
Day 2-5	189,8807	30	30,60281805	0,979906065	
Day 2-6	187,0245	30	30,13906987	0,995364338	
Day 3-1	182,9344	30	29,47497897	1,017500701	Average:
Day 3-2	184,4544	30	29,72177444	1,009274185	101%
Day 3-3	184,0031	30	29,64849892	1,011716703	Variance:
Day 3-4	187,396	30	30,19938863	0,993353712	0,000192212
Day 3-5	179,7289	30	28,95451654	1,034849449	
Day 3-6	185,3032	30	29,85959023	1,004680326	

6,158946032 1,399595238 **Average recovery:** 101%

Cochran's C test: study of the homogeneity of a series of v m : number of days for 1 test: 6
s² max 0,000459909 p: number of tests for validation: 3
sum s² 0,000728705

Cochran's critique C, α = 5% 0,7071

C test 0,631131657

C test < C 5% : The variances are homogeneous (No rejection of H0)

Estimation of repeatability

Average calculation: 101%
s² intra : 0,000242902
SD repeatability: 0,015500838
 1,55%

Estimation of intermediate precision

Average calculation: 101%
 Average s² : 3,86616E-08
s²IG : 2,3197E-07
s² facteur : -4,0445E-05
s²Rl: 0,000202457
SD intermediate precision: 0,014151615
 1,42%

Precision CLONIDINE within the mixture

Essay Number	AUC (mAu*min)	Concentration (µg/mL)	Observed concentration (µg/mL)	Recovery Rate	
Day 1-1	182,5687	30	28,77163949	1,04094535	Average:
Day 1-2	191,0772	30	30,14105531	0,995298156	100%
Day 1-3	188,7007	30	29,75856524	1,008047825	Variance:
Day 1-4	195,1747	30	30,8005348	0,973315507	0,000577868
Day 1-5	192,6216	30	30,38962154	0,987012615	
Day 1-6	193,2537	30	30,49135601	0,983621466	
Day 2-1	192,2494	30	30,32971714	0,989009429	Average :
Day 2-2	188,8567	30	29,78367294	1,007210902	100%
Day 2-3	193,123	30	30,47032026	0,984322658	Variance :
Day 2-4	191,8215	30	30,26084801	0,991305066	0,000122519
Day 2-5	189,445	30	29,87835794	1,004054735	
Day 2-6	188,0835	30	29,65922888	1,011359037	
Day 3-1	184,2875	30	29,04827484	1,031724172	Average:
Day 3-2	188,6166	30	29,74502962	1,008499013	103%
Day 3-3	187,8153	30	29,61606295	1,012797902	Variance:
Day 3-4	174,2105	30	27,42641398	1,085786201	0,000976677
Day 3-5	188,3177	30	29,69692262	1,010102579	
Day 3-6	189,7109	30	29,9211537	1,00262821	

6,21323333 3,803790476 **Average recovery:** 101%

Cochran's C test: study of the homogeneity of v m : number of days for 1 test: 6
s² max 0,000976677 p: number of tests for validation: 3
sum s² 0,001677065

Cochran's critique C, α = 5% 0,7071

C test 0,582373048

C test < C 5% : The variances are homogeneous (No rejection of H0)

Estimation of repeatability

Average calculation: 101%
s² intra : 0,000559022
SD repeatability: 0,02356064
 2,36%

Estimation of intermediate precision

Average calculation: 101%
 Average s² : 1,82663E-07
s²IG : 1,09598E-06
s² facteur : -9,29876E-05
s²Rl: 0,000466034
SD intermediate precision: 0,021512039
 2,15%

Accuracy
CLONIDINE drug product

Essay	Observed AUC (mg/h*min)	Concentration (mg/L)	observed concentration (mg/L)	Recovery (%)	Variance of recoveries	Average recovery
Day 1 15%	238.4622	23	20.4267509	99.2408184		
Day 2 25%	127.2703	23	20.4202189	97.2923044		
Day 3 15%	132.7258	23	11.6366613	100.7607893	3.3292403	98.7634851
Day 1 50%	149.0511	24	23.9720236	99.8999915		
Day 2 50%	146.7361	24	24.2677789	100.1849913		
Day 4 50%	151.2029	24	24.2027792	100.1451131	0.5456081	100.5266053
Day 1 100%	170.1317	27	27.2905355	100.4898413		
Day 2 100%	166.4511	27	26.9741336	99.2422759	1.382279641	100.1172362
Day 3 100%	192.1297	27	26.9232324	99.2086186		
Day 3 125%	198.9264	30	30.4424269	100.4488424		
Day 2 125%	187.757	30	30.2649717	100.8618572	0.108579683	100.2684558
Day 1 150%	202.9627	33	32.7268827	98.1728729		
Day 3 150%	198.7895	30	30.4554481	100.4286625		
Day 2 150%	202.9627	33	32.7268827	98.1728729		
Day 1 200%	222.0546	33	33.2288268	98.2288268		
Day 2 200%	206.6597	33	33.2518741	100.8811114	1.804753919	
Day 3 200%	222.0546	36	35.8432921	99.7923936		
Day 2 250%	232.1754	36	35.8049702	99.9718798		
Day 1 250%	224.9177	36	36.2816897	100.8105446	0.444622402	100.0478964
Day 2 250%	232.1754	36	36.2816897	100.8105446		
Day 1 250%	218.8034	39	38.5831729	98.8164433	1.142144019	99.7445518
Day 3 250%	243.8024	39	39.3978895	100.2172512	6.64802772	100.0733823
			Sum of variances			1.109429219
						Average of recovery averages
						1.109429219
						Total standard deviation of the recovery
6.15884632	1.39919238					

Cochran's C test C(7,3)

C test	0.37022794
C threshold (alpha)	0.861
p max	3.207903
chi sum	8.6480072

C test < C table, no rejection of H0, the variances are homogeneous at the 5% risk.

ANOVA

	Sum of SES	DOF	F	Fexp	p-value
Intragroup	17.32922154	14	1.23787253		
Intergroup	11.44839068	5	2.28678135	1.84978082	0.16756191
Total	28.77761222	19			

SES : Sum of standard errors squared

Mean Recovery Confidence Interval

Average R:	99.9811163
Total variance:	1.514637485
Number of DDF:	n-2 = 17
5%_2D DDF:	2.085963447
Lower bound:	99.1302693
Upper bound:	100.8459699

In the mean overlap confidence interval is : [99.13 ; 100.84]
The average recovery rate is 99.9831
It is included in the confidence interval: ok.

Accuracy
CLONIDINE within the mixture

Essay	Observed AUC (mg/h*min)	Concentration (mg/L)	observed concentration (mg/L)	Recovery (%)	Variance of recoveries	Average recovery
Day 1 15%	114.9375	23	21.0206097	100.494957		
Day 2 25%	134.3452	23	21.0202185	100.048145		
Day 3 25%	135.5624	23	20.8423445	99.4949514	0.371197142	99.9172806
Day 1 50%	151.8987	24	23.83582124	99.31471845		
Day 2 50%	152.7793	24	23.8488824	99.3687810		
Day 3 50%	154.6131	24	24.2727235	101.1444803	0.89161088	100.0718805
Day 1 100%	168.9953	27	26.5378702	98.29129446		
Day 2 100%	173.8201	27	27.30399195	101.3469919		
Day 3 100%	170.4483	27	26.8209984	99.3266664	2.410683862	99.65812159
Day 1 125%	198.2571	30	30.0561818	100.081238		
Day 2 125%	191.4522	30	30.20141035	100.6713678	1.212695408	99.7373665
Day 3 125%	187.4468	30	29.5162526	98.52192512		
Day 1 150%	206.9355	33	32.65337389	99.0706068		
Day 2 150%	211.3748	33	33.2288268	101.889189		
Day 3 150%	210.7729	33	33.0102519	100.9421701	1.266531775	
Day 1 200%	229.7489	36	36.3851544	101.642373		
Day 2 200%	228.9088	36	36.19996925	100.8479445		
Day 3 200%	229.1943	36	36.27588344	100.7963345	0.054635899	100.7578721
Day 1 250%	244.3243	39	38.7462512	98.4779272		
Day 2 250%	247.9516	39	39.2488778	100.7599174		
Day 3 250%	242.279	39	38.2109155	98.00100445	1.897702032	99.36423585
			Sum of variances			8.016612707
						99.9349118
						Average of recovery averages
						1.002194416
						Total standard deviation of the recovery
6.21223333	3.80379076					

Cochran's C test C(7,3)

C test	0.8077103
C threshold (alpha)	0.861
p max	2.410683862
chi sum	8.016612707

C test < C table, no rejection of H0, the variances are homogeneous at the 5% risk.

ANOVA

	Sum of SES	DOF	F	Fexp	p-value
Intragroup	16.0322241	14	1.14320387		
Intergroup	3.80959631	5	0.79619126	0.69522373	0.65098181
Total	20.0018204	19			

SES : Sum of standard errors squared

Mean Recovery Confidence Interval

Average R:	99.99672867
Total variance:	1.053378902
Number of DDF:	n-2 = 17
5%_2D DDF:	2.085963447
Lower bound:	99.44384752
Upper bound:	100.5495998

In the mean overlap confidence interval is : [99.44 ; 100.54]
The average recovery rate is 99.9831
It is included in the confidence interval: ok.