

LINEARITY

LOXAPINE within the mixture						
Essay	Observed AUC (mAu)	Concentration (µg/L)	Variance s²	Estimated AUC	Adjusted AUC	Confidence interval
D1 25%	6.035308681	5	0.405964319	1.044590036	6.035305881	0.769428901
D2 25%	6.016582598	5		1.044590036	6.035305881	0.757576342
D3 25%	7.129407403	5		1.044590036	7.129407403	0.937135769
D1 50%	28.79663711	10	3.047761949	22.09685315	28.79663711	1.020837434
D2 50%	29.31139338	10		22.09685315	29.31139338	1.099270184
D3 50%	26.0632709	10		22.09685315	26.0632709	0.604357862
D1 100%	58.39875211	20	0.079454578	64.20137938	58.39875211	-0.884138577
D2 100%	58.0362295	20		64.20137938	58.0362295	-0.938858873
D3 100%	57.84284537	20		64.20137938	57.84284537	-0.968841619
D1 125%	81.19942166	25	17.40136048	85.25364249	81.19942166	-0.617738695
D2 125%	78.86340892	25		85.25364249	78.86340892	-0.97367104
D3 125%	73.09517965	25		85.25364249	73.09517965	-1.852569289
D1 150%	99.80872165	30	6.045077926	106.3059056	103.8483443	-0.374455451
D2 150%	99.80872165	30		106.3059056	99.80872165	-0.9899601
D3 150%	99.40029141	30		106.3059056	99.40029141	-1.052199522
D1 200%	151.2165161	40	11.37442323	148.4104318	151.2165161	0.427559438
D2 200%	148.9022385	40		148.4104318	148.9022385	0.074939346
D3 200%	144.5724511	40		148.4104318	144.5724511	-0.58478817
D1 250%	198.9540936	50	0.313521196	190.5149581	198.9540936	1.285860184
D2 250%	198.0750972	50		190.5149581	198.0750972	1.151928651
D3 250%	197.9136897	50		190.5149581	197.9136897	1.127335186

Linear regression (LINEST function)			
Slope b	4.210452623	-20.00767308	Intercept a
S slope	0.087344407	2.879428759	S intercept
R²	0.989987071	6.563027309	S res
F	1878.546586	19	DOF
Regression sum of squares	80915.25223	818,3932216	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	0.450024745	k : nombre d'échantillons par essai
Cochran's critical C, α = 5%	0.561	C test = s'max/somme s²
Maximum S²	17.40136048	
Sum s²	38.66756365	
The variances are homogeneous at the risk of 0.05% because 0.4835<0.5685		

Calculation of experimental variance s²exp				
s²exp =	5.523397669			
Calculation of misfit variance				
SCE al =	763.153845			
s² al =	254.304615			
Fisher Test				
H0:	Not significantly different			
H1:	Significantly different			
F.exp.	46.05131887	F threshold 5% (5, 14)	2.958248913	p-value
Conclusion	p-value > 5%, therefore no rejection of H0: Fit to the validated linear model.			3,34971E-08

DEPENDENCE VERIFICATION. F(1, n - 2)				
H0:	The variances are not significantly different			
H1:	The variances are significantly different			
F.exp.	1878.546586	F threshold 5% (1, 19)	4.38	p-value
Conclusion	Dependence is validated as H0 is rejected, p-value=5%			1,84E-20

VALIDATION OF THE PROPORTIONAL MODEL				
Regression of LOXAPINE within the mixture: Comparison of the y-intercept to 0				
Intercept	20.00767308			
Standard deviation of the intercept Si	2.879428759			
Student Test				
H0:	The y-intercept is equal to 0			
H1:	The y-intercept is different from 0			
t exp	6.948486922			
Nb of DOF (N-2)	19			
t threshold (5%, student)	2.093			
Conclusion	texp > threshold→ Rejection of H0 at 5% risk. The y-intercept is significantly different from 0			
the correlation line does not pass through the origin				

LOXAPINE Pure drug Product Regression: Comparing the Intercept to 0				
Intercept	22.23347685			
Standard deviation of the in	2.67944703			
Student Test				
H0:	The y-intercept is equal to 0			
H1:	The y-intercept is different from 0			
t exp	8.29778555			
Nb of DOF (N-2)	19			
t threshold (5%, student)	2.093			
Conclusion	texp > threshold→ Rejection of H0 at 5% risk. The y-intercept is significantly different from 0			
the correlation line does not pass through the origin				

CALCULATED LIMIT OF DETECTION	15,58746267
CALCULATED LIMIT OF QUANTIFICATION	5,143862682

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

Comparison of Student slopes			
H0:	No difference between the two slopes		
H1:	Difference between the two slopes		
texp	0.085243682		
Nb of DOF (N1+N2-4)	38		
t threshold (5%, student à 38 dof)	2.042		
Conclusion	texp < t threshold → No rejection of H0 at 5% risk, no significant difference between the 2 slopes.		

COMPARISON OF STUDENT INTERCEPTS

H0:	No difference between the two intercepts		
H1:	Difference between the two intercepts		
t exp	0.565892644		
Nb of DOF (N1+N2-4)	38		
t threshold (5%, student à 38 dof)	2.042		
Conclusion	texp < t threshold → No rejection of H0 at 5% risk therefore no systematic error.		

SIGNIFICANCE TEST OF CLONIDINE IN THE MIXTURE FORM CORRELATION COEFFICIENT (Pearson correlation coefficient test)

H0:	R=0	R forme reconstituée:	0.99498094						
H1:	R different from 0								
texp	43.34220329								
Nb of DOF	19								
t threshold (5%, student)	2.093								
Conclusion	texp > t threshold → Rejection of H0 at 5% risk, existence of a significant linear link between the variables								

SIGNIFICANCE TEST OF CLONIDINE PURE PRODUCT CORRELATION COEFFICIENT (Pearson correlation coefficient test)

H0:	R=0	R pure product	0.995626195						
H1:	R different from 0								
texp	46.45193561								
Nb of DOF	19								
t threshold (5%, student)	2.093								
Conclusion	texp > t threshold → Rejection of H0 at 5% risk, existence of a significant linear link between the variables								

LINEARITY

LOXAPINE drug product						
Essay	Observed AUC (mAu)	Concentration (µg/L)	Variance s²	Estimated AUC	Adjusted AUC	Confidence interval
D1 25%	6.666106415	5	0.276074245	-1.237772056	6.666106415	1.294187511
D2 25%	5.670946023	5		-1.237772056	5.670946023	1.121190014
D3 25%	5.8768	5		-1.237772056	5.8768	1.16484835
D1 50%	19.97909416	10	1.410007363	19.75793274	19.97909416	0.036213152
D2 50%	20.58284873	10		19.75793274	20.58284873	0.135072418
D3 50%	22.2701	10		19.75793274	22.2701	0.411344318
D1 100%	56.13142117	20	0.01676441	61.74934233	56.13142117	-0.919882956
D2 100%	55.8868705	20		61.74934233	55.8868705	-0.959926487
D3 100%	55.9354	20		61.74934233	55.9354	-0.951979661
D1 125%	82.74504712	25	10.34456119	82.74504712	82.74504712	-0.868810134
D2 125%	75.42167693	25		82.74504712	75.42167693	-1.199134612
D3 125%	81.7201	25		82.74504712	81.7201	-0.167825678
D1 150%	98.82472467	30	10.14751669	103.7407519	98.82472467	-0.804564313
D2 150%	95.85896229	30		103.7407519	95.85896229	-1.290279835
D3 150%	102.225	30		103.7407519	102.225	-0.248190455
D1 200%	148.335377	40	34.70564574	145.7321615	148.335377	0.426252631
D2 200%	139.6769	40		145.7321615	139.6769	-0.99149346
D3 200%	150.9264	40		145.7321615	150.9264	0.850708851
D1 250%	196.6446373	50	45.40435749	187.7235711	196.6446373	1.460742659
D2 250%	186.0310594	50		187.7235711	186.0310594	-0.277133248
D3 250%	198.53	50		187.7235711	198.53	1.769453489

Linear regression (LINEST function)			
Slope b	4.199140959	-22.23347685	Intercept a
S slope	0.090397545	2.67944703	S intercept
R²	0.99127152	6.107212751	S res
F	2157.782322	19	DOF
Regression sum of squares	89481.06773	708,662904	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	0.443813986	p=5, n=3
Cochran's critical C, α = 5%	0.684	
Maximum S²	45.40435749	
Sum s²	102.3049271	
The variances are homogeneous at the risk of 0.05% because 0,341<0,684		

Calculation of experimental variance s²exp				
s²exp =	14.61498959			
Calculation of misfit variance				
SCE al =	562.5130081			
s² al =	187.504336			
Fisher Test				
H0:	Not significantly different			
H1:	Significantly different			
F.exp.	12.8295908	F threshold 5%(5,14)	2.958248913	p-value
Conclusion	p-value > 5%, therefore no rejection of H0: Fit to the validated linear model.			8,16657E-05

DEPENDENCE VERIFICATION. F(1, n - 2)				
H0:	The variances are not significantly different			
H1:	The variances are significantly different			
F.exp	2157.782322	F threshold 5% (1,19)	4.38	p-value
Conclusion	Dependence is validated as H0 is rejected, p-value=5%			4,98299E-21

Precision

LOXAPINE PUR DRUG

Essay Number	AUC (mAu*min)	Concentration (µg/mL)	Observed concentration (µg/mL)	Recovery Rate	
Day 1-1	85,20762243	25	25,5864474	98%	Average:
Day 1-2	81,62173369	25	24,73248971	101%	99%
Day 1-3	83,93600088	25	25,28361843	99%	Variance:
Day 1-4	83,82742447	25	25,25776161	99%	0,000196917
Day 1-5	85,59548421	25	25,67881434	97%	
Day 1-6	82,85592282	25	25,02640438	100%	
Day 2-1	82,97827174	25	25,05554103	100%	Average :
Day 2-2	80,01471794	25	24,34978863	103%	101%
Day 2-3	83,32224931	25	25,13745721	99%	Variance :
Day 2-4	80,32200797	25	24,42296789	102%	0,000259836
Day 2-5	84,00429621	25	25,29988255	99%	
Day 2-6	81,07740362	25	24,6028608	102%	
Day 3-1	84,26344762	25	25,36159789	99%	Average:
Day 3-2	83,4972141	25	25,17912401	99%	100%
Day 3-3	82,467397	25	24,9338793	100%	Variance:
Day 3-4	83,06564665	25	25,07634884	100%	5,80727E-05
Day 3-5	82,13577351	25	24,85490518	101%	
Day 3-6	82,43004033	25	24,92498304	100%	

4,199140959-22,23347685Average recovery:100%

Cochran's C test: study of the homogeneity of a series of variances C (m,p)		m : number of days for 1 test: 6
s² max	0,000259836	p: number of tests for validation: 3
sum s²	0,000514825	
Cochran's critique C, α = 5%	0,7071	
C test	0,504706567	
C test < C 5% : The variances are homogeneous (No rejection of H0)		

Estimation of repeatability	
Average calculation:	100%
s² intra :	0,000171608
SD repeatability:	0,013111087
	1,31%
Estimation of intermediate precision	
Average calculation:	100%
Average s² :	8,10143E-05
s²IG :	0,000486086
s² facteur :	5,24129E-05
s²RI:	0,000224021
SD intermediate precision:	0,014980078
	1,50%

Precision

LOXAPINE within the mixture

Essay Number	AUC (mAu*min)	Concentration (µg/mL)	Observed concentration (µg/mL)	Recovery Rate	
Day 1-1	84,20762243	25	25,34830346	99%	Average:
Day 1-2	83,25688062	25	25,12189005	100%	100%
Day 1-3	83,23485982	25	25,11664593	100%	Variance:
Day 1-4	83,82742447	25	25,25776161	99%	0,000131198
Day 1-5	81,90345761	25	24,79958055	101%	
Day 1-6	81,04857639	25	24,59599577	102%	
Day 2-1	81,92570238	25	24,80487801	101%	Average :
Day 2-2	82,94672905	25	25,04802933	100%	100%
Day 2-3	83,12694675	25	25,09094709	100%	Variance :
Day 2-4	80,32200797	25	24,42296789	102%	0,000156692
Day 2-5	84,12900063	25	25,32958015	99%	
Day 2-6	83,10474791	25	25,08566057	100%	
Day 3-1	84,26344762	25	25,36159789	99%	Average:
Day 3-2	81,12489362	25	24,61417025	102%	100%
Day 3-3	82,09378356	25	24,84490553	101%	Variance:
Day 3-4	82,06515852	25	24,83808865	101%	0,000209924
Day 3-5	83,49954091	25	25,17967813	99%	
Day 3-6	85,12490245	25	25,56674814	98%	

4,199140959-22,23347685Average recovery:100%

Cochran's C test: study of the homogeneity of a series of variances C (m,p)		m : number of days for 1 test: 6
s² max	0,000156692	p: number of tests for validation: 3
sum s²	0,000497813	
Cochran's critique C, α = 5%	0,7071	
C test	0,314759984	
C test < C 5% : The variances are homogeneous (No rejection of H0)		

Estimation of repeatability	
Average calculation:	100%
s² intra :	0,000165938
SD repeatability:	0,012887805
	2,40%
Estimation of intermediate precision	
Average calculation:	100%
Average s² :	4,63247E-06
s²IG :	2,77948E-05
s² facteur :	-2,30238E-05
s²RI:	0,000142914
SD intermediate precision:	0,011960343
	3,10%