

Linearity

MIDAZOLAM drug product

Essay	Observed AUC (mAu)	Concentration (µg/L)	Variance s ²	Estimated AUC	Adjusted AUC	Confidence interval
D1 25%	8.1053	5	0,486363773	4,941746889	8,1053	1,383407702
D2 25%	8.3127	5		4,941746889	8,3127	1,474102798
D3 25%	7,0145	5		4,941746889	7,0145	0,906405715
D1 50%	9,6464	6	0,182799543	8,759282901	9,6464	0,387932361
D2 50%	10,0161	6		8,759282901	10,0161	0,549600526
D3 50%	10,499	6		8,759282901	10,499	0,760770548
D1 100%	15,5306	8	0,262072243	16,39435493	15,5306	-0,377716187
D2 100%	16,4835	8		16,39435493	16,4835	0,038982744
D3 100%	16,3314	8		16,39435493	16,3314	-0,027529909
D1 125%	19,9329	10	4,66485841	24,02942695	19,9329	-1,791390011
D2 125%	21,2962	10		24,02942695	21,2962	-1,151498431
D3 125%	24,1843	10		24,02942695	24,1843	0,067725295
D1 150%	59,5402	20	4,23363828	62,20478708	59,5402	-1,165212074
D2 150%	63,6142	20		62,20478708	63,6142	0,616330001
D3 150%	61,0744	20		62,20478708	61,0744	-0,49431324
D1 200%	77,2558	25	1,758809443	81,29246714	77,2558	-1,765216582
D2 200%	79,6734	25		81,29246714	79,6734	-0,708010857
D3 200%	79,4095	25		81,29246714	79,4095	-0,823413153
D1 250%	177,0261	50	1,573380063	176,7308675	177,0261	0,129103888
D2 250%	179,5224	50		176,7308675	179,5224	1,220724765
D3 250%	178,4899	50		176,7308675	178,4899	0,769217108

Linear regression (LINEST function)			
Slope b	8,817936013	-14,14598317	Intercept a
S slope	0,033497984	0,775327669	S intercept
R ²	0,998539203	2,286782925	S res
F	12987,60127	19	DOF
Regression sum of squares	67917,0523	99,35814681	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	0,354420767	k : number of samples per test	C test = max s ² /sum s ²
Cochran's critical C, α = 5%	0,561		
Maximum S ²	4,66485841		
Sum s ²	13,16192176		

The variances are homogeneous at the risk of 0.05% because 0.354<0.561

Calculation of experimental variance s ² exp			
s ² exp =	1,880274537		
Calculation of misfit variance			
SCE ai =	80,55540144		
s ² ai =	26,85180048		
Fisher Test			
H0:	Not significantly different		
H1:	Significantly different		
F exp.	14,28078717	F THRESHOLD 5% (5, 14)	2,958248913
Conclusion	p-value > 5%, therefore no rejection of H0: Fit to the validated linear model.	p-value	4,49065605

DEPENDENCE VERIFICATION. F(1, n - 2)

The variances are not significantly different			
H0:	The variances are significantly different		
H1:	The variances are not significantly different		
F exp.	12987,60127	F threshold 5% (1, 19)	4,38
Conclusion	Dependence is validated as H0 is rejected, p-value<5%	p-value	2,09287628

VALIDATION OF THE PROPORTIONAL MODEL

Regression of midazolam within the mixture: Comparing the y-intercept to 0		Pure drug Product Regression: Comparing the intercept to 0	
tat	14,14593317	tat	8,658878536
Standard deviation of the intercept Sa	0,775327669	standard deviation	0,231653253
Student Test.		Student Test.	
H0:	L'ordonnée à l'origine est égale à 0	H0:	L'ordonnée à l'origine est égale à 0
H1:	L'ordonnée à l'origine est différente de 0	H1:	L'ordonnée à l'origine est différente de 0
t exp	18,24510299	t exp	37,37861829
Nb of DOF (N-2)	19	Nb of DOF (N-2)	19
t threshold (5%, student)	2,093	t threshold (5%, student)	2,093
Conclusion	texp > threshold → Rejection of H0 at 5% risk. The y-intercept is significantly different from 0	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		the correlation line does not pass through the origin

CALCULATED LIMIT OF DETECTION	5,990206557
CALCULATED LIMIT OF QUANTIFICATION	1,976768164

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	12,73570059		
Nb of DOF (N1+N2-4)	38		
t threshold (5%, student à 38 dof)	2,042		
Conclusion	texp > t threshold → Rejection of H0 at 5% risk, significant difference between the 2 slopes		

COMPARISON OF STUDENT INTERCEPTS

No difference between the two intercepts			
H0:	Difference between the two intercepts		
H1:	No difference between the two intercepts		
F exp	6,780882227		
Nb of DOF (N1+N2-4)	38		
t threshold (5%, student à 38 dof)	2,042		
Conclusion	texp > t threshold → rejection of H0 at 5% risk, therefore systematic error		

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

R recomstituted form: 0,999269335			
H0:	R=0		
H1:	R different from 0		
texp	113,9631575		
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 MIDAZOLAM PURE PRODUCT CORRELATION COEFFICIENT (Pearson correlation coefficient test)

R pure product: 0,999947635			
H0:	R=0		
H1:	R different from 0		
texp	425,9143808		
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

MIDAZOLAM within the mixture

Essay	Observed AUC (mAu)	Concentration (µg/L)	Variance s ²	Estimated AUC	Adjusted AUC	Confidence interval
D1 25%	11,9606	5	0,081154943	12,65507912	11,9606	-1,01643855
D2 25%	12,5097	5		12,65507912	12,5097	-0,212776647
D3 25%	12,3668	5		12,65507912	12,3668	-0,421924864
D1 50%	16,7904	6	0,154529053	16,91787065	16,7904	-0,186565838
D2 50%	16,1378	6		16,91787065	16,1378	-1,141710181
D3 50%	16,8438	6		16,91787065	16,8438	-0,108409681
D1 100%	25,0072	8	0,410336143	25,44345371	25,0072	-0,638500244
D2 100%	26,1641	8		25,44345371	26,1641	1,054736792
D3 100%	25,109	8		25,44345371	25,109	-0,489595923
D1 125%	34,2853	10	0,02863164	33,96903677	34,2853	0,46282375
D2 125%	34,1191	10		33,96903677	34,1191	0,219621214
D3 125%	34,4575	10		33,96903677	34,4575	0,714914027
D1 150%	75,9421	20	0,278535303	76,59695207	75,9421	-0,958440467
D2 150%	76,9176	20		76,59695207	76,9176	0,469399812
D3 150%	76,0807	20		76,59695207	76,0807	-0,755585724
D1 200%	99,4188	25	0,693013463	97,91090972	99,4188	2,206945862
D2 200%	98,0661	25		97,91090972	98,0661	0,227136251
D3 200%	99,5831	25		97,91090972	99,5831	2,447415086
D1 250%	204,1925	50	0,01606396	204,480698	204,1925	-0,42180611
D2 250%	204,0261	50		204,480698	204,0261	-0,66534889
D3 250%	203,9437	50		204,480698	203,9437	-0,785949402

Linear regression (LINEST function)			
Slope b	4,26279153	-8,638878536	Intercept a
S slope	0,010008564	0,231653253	S intercept
R ²	0,999895272	0,683247517	S res
F	181403,0598	19	DOF
Regression sum of squares	84683,87682	8,869716209	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	0,416909409	k : number of samples per test	C test = max s ² /sum s ²
Cochran's critical C, α = 5%	0,561		
Maximum S ²	0,693013463		
Sum s ²	1,66293907		

The variances are homogeneous at the risk of 0.05% because 0.417<0.561

Calculation of experimental variance s ² exp			
s ² exp =	0,237466272		
Calculation of misfit variance			
SCE ai =	6,495053485		
s ² ai =	2,165017828		
Fisher Test			
H0:	Not significantly different		
H1:	Significantly different		
F exp.	9,117159277	F THRESHOLD 5% (5, 14)	2,958248913
Conclusion	p-value > 5%, therefore no rejection of H0: Fit to the validated linear model.	p-value	0,00049823

DEPENDENCE VERIFICATION. F(1, n - 2)

The variances are not significantly different			
H0:	The variances are significantly different		
H1:	The variances are not significantly different		
F exp.	181403,0598	F threshold 5% (1, 19)	4,38
Conclusion	Dependence is validated as H0 is rejected, p-value<5%	p-value	2,80205639

Precision

MIDAZOLAM drug product

Essay Number	AUC (mAu*min)	Concentration (µg/mL)	Observed concentration (µg/mL)	Recovery Rate	
D1-1	75,7118	25	23,4240767	94%	Average :
D1-2	77,5101	25	23,89090771	96%	Variance: 94%
D1-3	75,7042	25	23,42210377	94%	Average :
D2-1	76,7345	25	23,68956526	95%	Variance: 96%
D2-2	80,9352	25	24,78004908	99%	Average :
D2-3	76,7043	25	23,68172547	95%	Variance: 96%
D3-1	79,3289	25	24,36306045	97%	Average :
D3-2	78,1888	25	24,06709534	96%	Variance: 97%
D3-3	79,5651	25	24,42437696	98%	Average :
3,852143345		-14,52110116	Average recovery:		96%

0,000116723

0,000638808

5,8401E-05

Cochran's C test: study of the homogeneity of a series of variances C (m,p)

s^2 max 0,000638808 m : number of days for 1 test: 6
 sum s^2 8,1393E-04 p : number of tests for validation: 3
 Cochran's critic C, $\alpha = 5\%$ 0,7071

C test 7,8484E-01

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

Estimation of repeatability

Average calculation: 96%
 s^2 intra : 2,7131E-04
 SD repeatability: 0,016821187
 1,68%

Estimation of intermediate precision

Average calculation: 96%
 Average s^2 : 8,64965E-08
 s^2 IG : 5,18979E-07
 s^2 factor : -4,5132E-05
 s^2 RI: 0,000226179
 SD intermediate precision: 0,015358509
 1,54%

Precision

MIDAZOLAM within the mixture

Essay Number	AUC (mAu*min)	Concentration (µg/mL)	Observed concentration (µg/mL)	Recovery Rate	
D1-1	98,5854	25	25,15822737	0,993670905	Average :
D1-2	98,7812	25	25,20415971	0,991833612	Variance: 99%
D1-3	98,7416	25	25,19487002	0,992205199	Average :
D1-4	97,1472	25	24,82084282	1,007166287	Variance: 7,457E-05
D1-5	97,9952	25	25,01977349	0,99920906	Average :
D1-6	99,9176	25	25,47074558	0,981170177	Variance: 99%
D2-1	98,4846	25	25,13458089	0,994616764	Average :
D2-2	99,4556	25	25,3623659	0,985505364	Variance: 99%
D2-3	97,9804	25	25,01630159	0,999347936	Average :
D2-4	98,7816	25	25,20425354	0,991829858	Variance: 2,8975E-05
D2-5	97,8891	25	24,9948837	1,000204652	Average :
D2-6	98,4027	25	25,11536813	0,995385275	Variance: 99%
D3-1	99,7635	25	25,43459556	0,982616178	Average :
D3-2	98,2531	25	25,08027375	0,99678905	Variance: 99%
D3-3	98,4641	25	25,12977184	0,994809127	Average :
D3-4	98,0469	25	25,0319017	0,998723932	Variance: 5,5456E-05
D3-5	98,9687	25	25,24814497	0,990074201	Average :
D3-6	97,4677	25	24,89602829	1,004158868	Variance: 99%
4,26279153		-8,658878536	Average recovery:		99%

Cochran's C test: study of the homogeneity of a series of variances C (m,p)

s^2 max 7,457E-05 m : number of days for 1 test: 6
 sum s^2 0,000159001 p : number of tests for validation: 3
 Cochran's critic C, $\alpha = 5\%$ 0,7071

C test 0,468992156

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

Estimation of repeatability

Average calculation: 99%
 s^2 intra : 5,30002E-05
 SD repeatability: 0,007300569
 0,73%

Estimation of intermediate precision

Average calculation: 99%
 Average s^2 : 5,24253E-10
 s^2 IG : 3,14552E-09
 s^2 factor : -8,83284E-06
 s^2 RI: 4,41673E-05
 SD intermediate precision: 0,006664517
 0,67%

