

Linearity

SUFENTANIL drug product						
Essay	Observed AUC (mAu)	Concentration (µg/L)	Variance s²	Estimated AUC	Adjusted AUC	Confidence interval
70% D1	29,9176	0,7	0,113118493	29,77485119	29,9176	0,2291513636
	29,397	0,7		29,77485119	29,397	-0,606561768
	29,2884	0,7		29,77485119	29,2884	-0,780896558
70% D2	32,3645	0,8	0,76291591	31,21847381	32,3645	1,839707508
70% D3	30,6921	0,8		31,21847381	30,6921	-0,844984048
80% D1	31,0912	0,8		31,21847381	31,0912	-0,204311721
80% D2	33,5192	0,9	0,494723703	32,66209643	33,5192	1,375902129
80% D3	32,3083	0,9		32,66209643	32,3083	-0,567946833
90% D1	32,2937	0,9		32,66209643	32,2937	-0,591384107
90% D2	34,1135	1	0,010401943	34,10571905	34,1135	0,012490706
90% D3	34,2246	1		34,10571905	34,2246	0,190838728
100% D1	34,3172	1		34,10571905	34,3172	0,339468835
100% D2	36,1929	1,1	0,281481943	35,54934167	36,1929	1,033099512
100% D3	35,5591	1,1		35,54934167	35,5591	0,015664982
110% D1	35,139	1,1		35,54934167	35,139	-0,658718493
110% D2	38,0203	1,2	1,029620653	36,99296429	38,0203	1,64917455
110% D3	36,7763	1,2		36,99296429	36,7763	-0,347809602
120% D1	36,0097	1,2		36,99296429	36,0097	-1,578427006
120% D2	39,3044	1,3	0,813711143	38,4365869	39,3044	1,39309405
120% D3	38,1685	1,3		38,4365869	38,1685	-0,430357958
130% D1	37,5226	1,3		38,4365869	37,5226	-1,46721653

Linear regression (LINEST function):			
Slope b	14,43622619	19,66949286	Intercept a
S slope	0,679602548	0,693142915	S intercept
R²	0,959585058	0,622939345	S res
F	451,1231542	19	DOF
Regression sum of squares	175,0598864	7,373015129	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	0,293676084	k : number of samples per test	
Cochran's critical C, α = 5%	0,561	C test = max s²/sum s²	
Maximum S²	1,029620653		0,293676084
Sum s²	3,50597379		
The variances are homogeneous at the risk of 0.05% because 0.294<0.561			

Linearity

SUFENTANIL within the mixture						
Essay	Observed AUC (mAu)	Concentration (µg/L)	Variance s²	Estimated AUC	Adjusted AUC	Confidence interval
70% D1	32,5024	0,7	0,19813996	33,13350595	32,5024	-1,525704847
70% D2	33,047	0,7		33,13350595	33,047	-0,209128891
70% D3	33,3846	0,7		33,13350595	33,3846	0,60702233
80% D1	34,4409	0,8	0,766438013	34,57480238	34,4409	-0,323710323
80% D2	34,1067	0,8		34,57480238	34,1067	-1,13164211
80% D3	35,1197	0,8		34,57480238	35,1197	1,31729354
90% D1	36,0963	0,9	0,259439053	36,01609881	36,0963	0,193887167
90% D2	35,2057	0,9		36,01609881	35,2057	-1,959147093
90% D3	36,0793	0,9		36,01609881	36,0793	0,1527895
100% D1	38,2199	1	0,08411863	37,45739524	38,2199	1,84362762
100% D2	37,8916	1		37,45739524	37,8916	1,049694283
100% D3	37,6416	1		37,45739524	37,6416	0,445316824
110% D1	39,1214	1,1	0,051236943	38,89869167	39,1214	0,78015057
110% D2	39,3788	1,1		38,89869167	39,3788	1,160666618
110% D3	38,9325	1,1		38,89869167	38,9325	0,081731978
120% D1	40,4748	1,2	0,025822813	40,3399881	40,4748	0,325909106
120% D2	40,1538	1,2		40,3399881	40,1538	-0,450111552
120% D3	40,328	1,2		40,3399881	40,328	-0,28981138
130% D1	41,4144	1,3	0,03945499	41,78128452	41,4144	-0,88646845
130% D2	41,6776	1,3		41,78128452	41,6776	-0,250658356
130% D3	41,2883	1,3		41,78128452	41,2883	-1,191794935

Linear regression (LINEST function):			
Slope b	14,41296429	23,04443095	Intercept a
S slope	0,451327823	0,460265875	S intercept
R²	0,981705971	0,413648782	S res
F	1019,817374	19	DOF
Regression sum of squares	174,4961732	3,251000988	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	0,28815		
Cochran's critical C, α = 5%	0,561		
Maximum S²	0,266438013		
Sum s²	0,924650403		
The variances are homogeneous at the risk of 0.05% because 0.288<0.561			

<u>calculation of experimental variance s²exp</u>			
<u>s²exp</u> =	0,500853399		
<u>calculation of misfit variance</u>			
<u>SCE aj</u> =	2,364481144		
<u>s² aj</u> =	0,788160381		
<u>Fisher Test</u>			
<u>H0:</u>	Not significantly different		
<u>H1:</u>	Significantly different		
<u>Fexp</u>	1,573634887	<u>F seuil 5% (5, 14)</u>	2,958248913
		<u>p-value</u>	0,231059692
<u>Conclusion</u>	p-value > 5%, therefore no rejection of H0: Fit to the validated linear model.		

<u>DEPENDENCE VERIFICATION. F(1, n - 2)</u>			
<u>H0:</u>	The variances are not significantly different		
<u>H1:</u>	The variances are significantly different		
<u>F exp</u>	451,1231542	<u>F threshold 5% (1, 19)</u>	4,38
		<u>p-value</u>	1,06437E-14
<u>Conclusion</u>	Dependence is validated as H0 is rejected, p-value<5%		

Calculation of experimental variance s^2_{exp}			
$s^2_{exp} =$	0,132092915		
Calculation of misfit variance			
$SCE_{aj} =$	1,930071841		
$s^2_{aj} =$	0,64335728		
Fisher Test			
$H_0:$	Not significantly different		
$H_1:$	Significantly different		
F_{exp}	4,870490453	F seuil 5% (5, 14)	2,958248913 p-value
Conclusion	p-value > 5%, therefore no rejection of H_0 : Fit to the validated linear model.		
			0,008645393
DEPENDENCE VERIFICATION. F(1, n - 2)			
$H_0:$	The variances are not significantly different		
$H_1:$	The variances are significantly different		
F_{exp}	1019,817374	F threshold 5% (1, 19)	4,38
Conclusion	Dependence is validated as H_0 is rejected, p-value<5%		
			5,64303E-18

VALIDATION OF THE PROPORTIONAL MODEL

Regression of SUFENTANIL within the mixture: Comparison of the y-intercept to 0			
lal	19,66949286		
Standard deviation of the intercept	0,693142915		
Student Test			
H0:	The y-intercept is equal to 0		
H1:	The y-intercept is different from 0		
t exp	28,37725442		
Nb of DOF (N-2)	19		
t threshold (5%, student)	2,093		
Conclusion	texp > t threshold → reject of H0 at 5% risk. The y-intercept is significantly different from 0		
Conclusion	the correlation line doesn't pass through the origin		

SUFENTANIL Pure drug Product Regression: Comparing the Intercept to 0			
lal	23,04443095		
Standard deviation	0,460265875		
Student Test			
H0:	The y-intercept is equal to 0		
H1:	The y-intercept is different from 0		
t exp	50,06765044		
Nb of DOF (N-2)	19		
t threshold (5%, student)	2,093		
Conclusion	texp > t threshold → reject of H0 at 5% risk. The y-intercept is significantly different from 0		
Conclusion	the correlation line doesn't pass through the origin		

CALCULATED LIMIT OF DETECTION	0,431511211
CALCULATED LIMIT OF QUANTIFICATION	0,1423987

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	0,028511322	
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	4,056217408	
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 SUFENTANIL IN THE MIXTURE FORM CORRELATION COEFFICIENT (Pearson correlation coefficient test)

H0:	R=0	R reconstituted form:	5,684039057
H1:	R different from 0		
texp	123,2431739		
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 SUFENTANIL PURE PRODUCT CORRELATION COEFFICIENT (Pearson correlation coefficient test)

H0 :	R=0	R pure product	0,990812783
H1 :	R different from 0		
texp	31,93457959		
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		

Precision

SUFENTANIL drug product

Essay Number	AUC (mAu*min)	Concentration (µg/mL)	Observed concentration (µg/mL)	Recovery Rate	
Day 1-1	34,5353	1	1,02975715	0,97024285	Average:
Day 1-2	34,0439	1	0,995717783	1,004282217	102%
Day 1-3	33,9958	1	0,992385888	1,007614112	Variance:
Day 1-4	33,3699	1	0,949029681	1,050970319	0,000976774
Day 1-5	33,4586	1	0,955173946	1,044826054	
Day 1-6	33,5168	1	0,959205471	1,040794529	
Day 2-1	33,4472	1	0,954384266	1,045615734	Average :
Day 2-2	33,3014	1	0,944284674	1,055715326	104%
Day 2-3	33,4063	1	0,951551116	1,048448884	Variance :
Day 2-4	33,9534	1	0,989448832	1,010551168	0,000292599
Day 2-5	33,5224	1	0,959593384	1,040406616	
Day 2-6	33,2782	1	0,942677606	1,057322394	
Day 3-1	33,5635	1	0,962440388	1,037559612	Average:
Day 3-2	34,1702	1	1,004466607	0,995533393	100%
Day 3-3	34,2548	1	1,010326865	0,989673135	Variance:
Day 3-4	33,8978	1	0,98559741	1,01440259	0,00039867
Day 3-5	34,1569	1	1,003545314	0,996454686	
Day 3-6	34,3486	1	1,016824408	0,983175592	

14,4362261919,66949286Average recovery:102%

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

s² max0,000976774
sum s²0,001668043
Cochran's critique C, α = 5%0,7071
C test0,585580916

m : number of days for 1 test: 6
p: number of tests for validation: 3

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

Estimation of repeatability

Average calculation:102%
s² intra :0,000556014
SD repeatability:0,0233263072,33%

Estimation of intermediate precision

Average calculation:102%
Average s² :1,35592E-07
s²IG :8,13551E-07
s² facteur : -9,25335E-05
s²RI:0,000463481
SD intermediate precision:0,0212970232,13%

Precision

SUFENTANIL within the mixture

Essay Number	AUC (mAu*min)	Concentration (µg/mL)	Observed concentration (µg/mL)	Recovery Rate	
Day 1-1	37,3353	1	0,991528791	1,008471209	Average:
Day 1-2	37,0607	1	0,972476499	1,027523501	105%
Day 1-3	36,6511	1	0,94405764	1,05594236	Variance:
Day 1-4	35,937	1	0,894511968	1,105488032	0,001182606
Day 1-5	36,7745	1	0,952619376	1,047380624	
Day 1-6	36,3756	1	0,924942904	1,075057096	
Day 2-1	37,0242	1	0,969944057	1,030055943	Average :
Day 2-2	37,3203	1	0,990488061	1,009511939	102%
Day 2-3	38,2879	1	1,057622065	0,942377935	Variance :
Day 2-4	36,7447	1	0,950551793	1,049448207	0,002313497
Day 2-5	37,5301	1	1,0050444	0,9949556	
Day 2-6	36,2738	1	0,917879819	1,082120181	
Day 3-1	38,2014	1	1,051620524	0,948379476	Average:
Day 3-2	37,9783	1	1,036141404	0,963858596	96%
Day 3-3	37,6075	1	1,010414566	0,989585434	Variance:
Day 3-4	37,6624	1	1,014223636	0,985776364	0,002144187
Day 3-5	39,1903	1	1,120232364	0,879767636	
Day 3-6	37,29147647	1	0,988488227	1,011511773	

14,4129642923,04443095Average recovery:101%

Cochran's C test: study of the homogeneity of a series of variances

s² max0,002313497
sum s²0,00564029
Cochran's critique C, α = 5%0,7071
C test0,410173401

m : number of days for 1 test: 6
p: number of tests for validation: 3

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

Estimation of repeatability

Average calculation:101%
s² intra :0,001880097
SD repeatability:0,043112644,31%

Estimation of intermediate precision

Average calculation:101%
Average s² :3,72036E-07
s²IG :2,23222E-06
s² facteur : -0,000312977
s²RI:0,001567119
SD intermediate precision:0,0393609483,94%

Accuracy

SUFENTANIL drug product

Essay	Observed AUC (mAu*min)	Concentration (mg/L)	observed concentration (mg/L)	Recovery rate (%)	Variance of recoveries	Average recovery
Day 1 25%	29,9176	0,7	0,709888236	101,4126051	11,07720456	97,61989779
Day 2 25%	29,397	0,7	0,67382618	96,26088281		
Day 3 25%	29,2884	0,7	0,666303438	95,18620543		
Day 1 50%	32,3645	0,8	0,879385442	109,9231802	57,19911927	101,4211314
Day 2 50%	30,6921	0,8	0,763537991	95,44224887		
Day 3 50%	31,0912	0,8	0,79118372	98,89796502		
Day 1 100%	33,5192	0,9	0,959371719	106,5968577	29,30693114	100,3461218
Day 2 100%	32,3083	0,9	0,875492457	97,27693971		
Day 3 100%	32,2937	0,9	0,874481113	97,16456808		
Day 1 125%	34,1135	1	1,000538988	100,0538988	0,499122476	100,7807739
Day2 125%	34,2246	1	1,008234905	100,8234905		
Day 3 125%	34,3172	1	1,014649324	101,4649324		
Day 1 150%	36,1929	1,1	1,144579402	104,0526729	11,16240599	100,5100279
Day 2 150%	35,5591	1,1	1,100675962	100,061451		
Day 3 150%	35,139	1,1	1,071575559	97,41595986		
Day 1 200%	38,0203	1,2	1,271163731	105,9303109	34,30894766	99,66790171
Day 2 200%	36,7763	1,2	1,184991626	98,74930214		
Day 3 200%	36,0097	1,2	1,131889105	94,32409209		
Day 1 250%	39,3044	1,3	1,36011357	104,6241208	23,10341625	99,44182317
Day 2 250%	38,1685	1,3	1,281429572	98,57150551		
Day 3 250%	37,5226	1,3	1,236687962	95,12984321		
Sum of variances				166,6571473	99,96966824	Average of recovery averages
					4,343152317	Total standard deviation of the recovery
14,43622619		19,66949286				

14,4362261919,66949286

Cochran's C test C(7,3)

C test	0,343214319
C threshold (cochran)	0,561
s²max	57,19911927
s² sum	166,6571473
C test < C table, no rejection of H0, the variances are homogeneous at the 5% risk.	

ANOVA

	Sum of SES	DOF	s²	Fexp	p-value
Intragroup	333,3142947	14	23,8081639	0,229067177	0,943563845
Intergroup	27,26834442	5	5,453668885		
Total	360,5826391	19			

SES : Sum of standard errors squared

Mean Recovery Confidence Interval

Average R:	99,96966824
total variance :	18,97803364
Number of DOF:	n-1 =20
t 5%, 20 DOF:	2,085963447
lower bound :	97,62335133
Upper bound :	102,3159852

So the mean overlap confidence interval is : [99,1487 ; 101,9262]
The average recovery rate is 100,5374
It is included in the confidence interval: ok.

Accuracy

SUFENTANIL within the mixture

Essay	Observed AUC [mAu*min]	Concentration (mg/L)	observed concentration (mg/L)	Recovery rate (%)	Variance of recoveries	Average recovery
Day 1 25%	32,5024	0,7	0,656212619	93,74465992	19,4656706	98,45866987
Day 2 25%	33,047	0,7	0,69398046	99,14257796		
Day 3 25%	33,3846	0,7	0,717421402	102,4887717		
Day 1 50%	34,4409	0,8	0,790709588	98,83869846	20,04054795	99,83490806
Day 2 50%	34,1067	0,8	0,76752213	95,94026625		
Day 3 50%	35,1197	0,8	0,837806076	104,7257595		
Day 1 100%	36,0963	0,9	0,905564517	100,6182797	15,41855631	98,2860173
Day 2 100%	35,2057	0,9	0,843772926	93,75254733		
Day 3 100%	36,0793	0,9	0,904385024	100,4872249		
Day 1 125%	38,2199	1	1,05290409	105,290409	4,049352368	103,1936856
Day2 125%	37,8916	1	1,030125986	103,0125986		
Day 3 125%	37,6416	1	1,012780491	101,2780491		
Day 1 150%	39,2214	1,1	1,122390143	102,0354676	2,0384085	101,7589909
Day 2 150%	39,3788	1,1	1,133310867	103,0282606		
Day 3 150%	38,9325	1,1	1,102345689	100,2132445		
Day 1 200%	40,4748	1,2	1,209353517	100,7794597	0,863245738	99,87787946
Day 2 200%	40,1538	1,2	1,187081901	98,92349177		
Day 3 200%	40,328	1,2	1,199168242	99,93068685		
Day 1 250%	41,4144	1,3	1,274544825	98,04190964	1,123850678	98,28581398
Day 2 250%	41,6776	1,3	1,292806162	99,44662788		
Day 3 250%	41,2883	1,3	1,265795758	97,36890443		
Sum of variances				62,99963214	99,95656645 Average of recovery averages	
					3,088064046 Total standard deviation of the recovery	
14,41296429		23,04443095				

14,4129642923,04443095

Cochran's C test C(7,3)

C test	0,318105793
C threshold (cochran table)	0,561
s²max	20,04054795
s² sum	62,99963214
C test < C table, no rejection of H0, the variances are homogeneous at the 5% risk.	

ANOVA

	Sum of SES	DOF	s²	Fexp	p-value
Intragroup	125,9992643	14	8,999947449	1,438308992	0,270926903
Intergroup	64,72352671	5	12,94470534		
Total	190,722791	19			

SES : Sum of standard errors squared

Mean Recovery Confidence Interval

Average R:	99,95656645
total variance :	10,03804163
Number of DOF:	n-1 =20
t 5%, 20 DOF:	2,085963447
lower bound :	98,25014791
Upper bound :	101,662985

So the mean overlap confidence interval is : [99,1487 ; 101,9262]
The average recovery rate is 100,5374
It is included in the confidence interval: ok.