Bioassay

Biológiai anyagok hatásának értékelése, ha közvetlen fizikai vagy kémiai analízis nem alkalmazható.

Alapja standard készítménnyel való összehasonlítás: a vizsgált anyag milyen mennyisége ad ugyanakkora hatást, mint a standard egysége azonos idő alatt azonos körülmények között.

Például a penicillin-készítménynek mekkora adagja ad ugyanakkora átmérőjű baktériumnövekedés-gátló zónát a táptalajon, mint a standard készítmény, vagy mekkora adag endotoxin-készítmény emeli meg ugyanannyival a nyulak testhőmérsékletét, mint a standard készítmény.

$F_T(z) = F_S(\rho z)$ similarity ρ relative potency

kvantitatív és kvantált

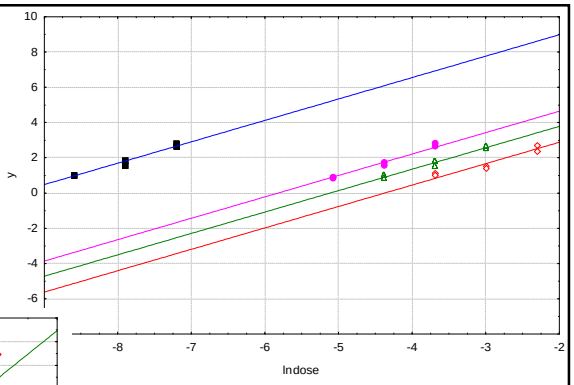
a kvantitatív kvantálttá butítható

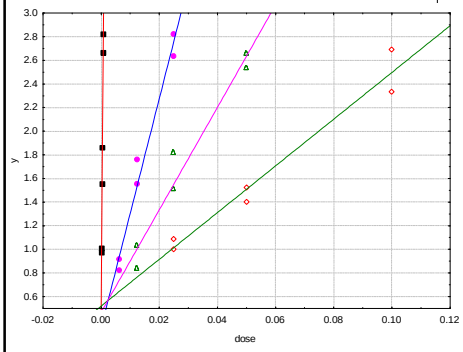
$F_T(z) = F_S(\rho z)$ similarity

A statisztikai hasonlóság nem bizonyítja, hogy nincs valóságos különbség, de nem-teljesülése azt jelzi, hogy valóságos különbség vagy mátrix-hatás van.

Parallel line assay

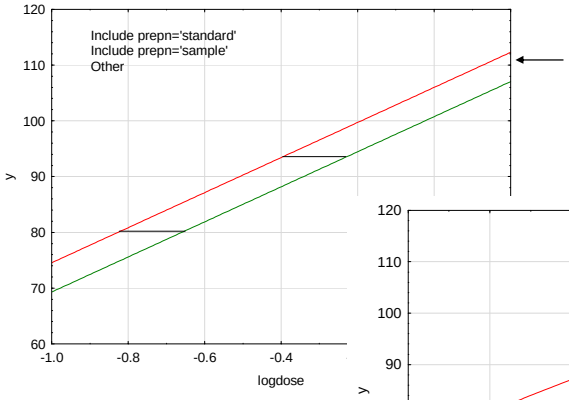
Párhuzamos egyenesek a dózis logaritmusa függvényében





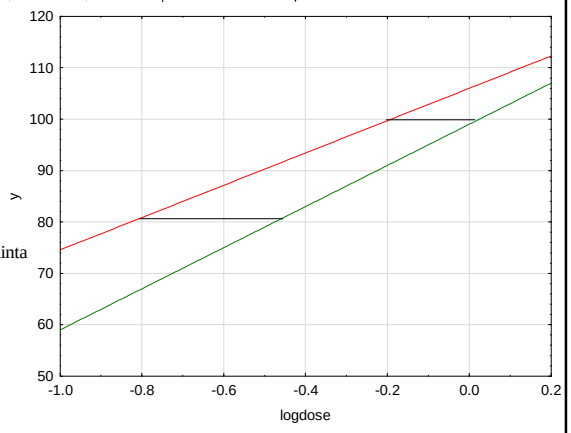
Slope ratio assay

Egy pontban találkozó egyenesek



ha párhuzamosak

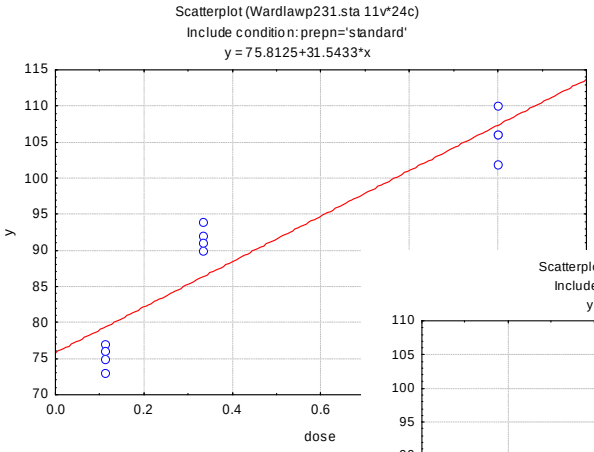
ha nem párhuzamosak



$$\lg c_{0\text{minta}} - \lg c_{0\text{std}} = \lg x_{\text{std}} - \lg x_{\text{minta}}$$

hol?

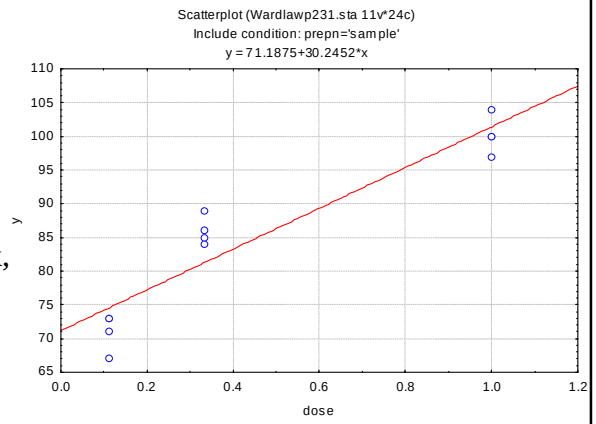
$$x = \frac{1}{\text{dilution}}$$



1. példa

A.C.Wardlaw: Practical statistics for experimental biologists, J.Wiley, 1985, p. 231

c_{std}=5NE/ml



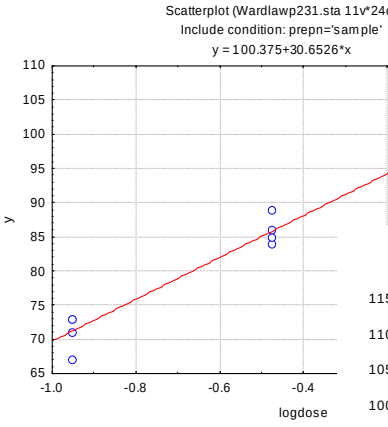
Parallel line assay a szándék, 6 pontos, hogy a linearitás vizsgálható legyen

	1 prepn	2 dilution	3 dose	4 logdose	5 rept	6 y
1	standard	9	0.111	-0.95424	1	77
2	standard	9	0.111	-0.95424	2	75
3	standard	9	0.111	-0.95424	3	76
4	standard	9	0.111	-0.95424	4	73
5	standard	3	0.333	-0.47712	1	92
6	standard	3	0.333	-0.47712	2	94
7	standard	3	0.333	-0.47712	3	90
8	standard	3	0.333	-0.47712	4	91
9	standard	1	1.000	0	1	110
10	standard	1	1.000	0	2	102
11	standard	1	1.000	0	3	106
12	standard	1	1.000	0	4	106
13	sample	9	0.111	-0.95424	1	73
14	sample	9	0.111	-0.95424	2	71
15	sample	9	0.111	-0.95424	3	73
16	sample	9	0.111	-0.95424	4	67
17	sample	3	0.333	-0.47712	1	84
18	sample	3	0.333	-0.47712	2	85
19	sample	3	0.333	-0.47712	3	86
20	sample	3	0.333	-0.47712	4	89
21	sample	1	1.000	0	1	100
22	sample	1	1.000	0	2	104
23	sample	1	1.000	0	3	97
24	sample	1	1.000	0	4	100

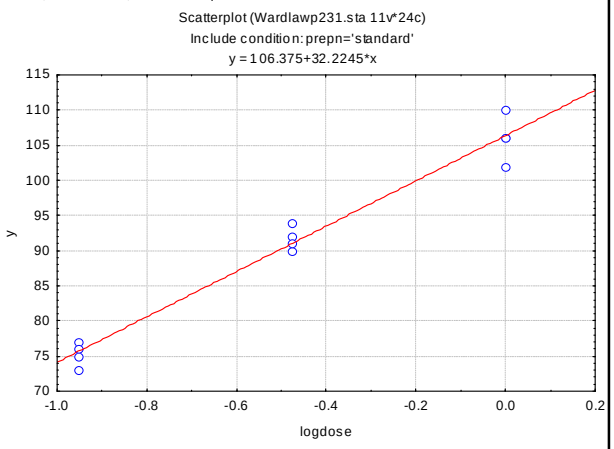
Dependnt Variable	Test of Lack of Fit (Wardlawp231.sta) Include condition: prepn='standard'							
	SS Pure Err	df Pure Err	MS Pure Err	SS Lack of Fit	df Lack of Fit	MS Lack of Fit	F	p
y	49.50000	9	5.500000	191.1635	1	191.1635	34.75699	0.000230

Dependnt Variable	Test of Lack of Fit (Wardlawp231.sta) Include condition: prepn='sample'							
	SS Pure Err	df Pure Err	MS Pure Err	SS Lack of Fit	df Lack of Fit	MS Lack of Fit	F	p
y	62.75000	9	6.972222	145.4712	1	145.4712	20.86439	0.001351

Két külön egyenes *y* vs. dózis nem adekvátak az egyenesek



a dózis logaritmusát véve (lg)



Test of Lack of Fit (Wardlawp231.sta)
Include condition: prepn='sample'

Dependnt Variable	SS Pure Err	df Pure Err	MS Pure Err	SS Lack of Fit	df Lack of Fit	MS Lack of Fit	F	p
y	62.75000	9	6.972222	0.375000	1	0.375000	0.053785	0.821791

Parameter Estimates (Wardlawp231.sta)
Sigma-restricted parameterization
Include condition: prepn='sample'

Effect	y Param.	y Std.Err	y t	y p
Intercept	100.3750	1.146780	87.52769	0.000000
logdose	30.6526	1.861774	16.46418	0.000000

Test of Lack of Fit (Wardlawp231.sta)
Include condition: prepn='standard'

Dependnt Variable	SS Pure Err	df Pure Err	MS Pure Err	SS Lack of Fit	df Lack of Fit	MS Lack of Fit	F	p
y	49.50000	9	5.500000	3.375000	1	3.375000	0.613636	0.453542

Parameter Estimates (Wardlawp231.sta)
Sigma-restricted parameterization
Include condition: prepn='standard'

Effect	y Param.	y Std.Err	y t	y p
Intercept	106.3750	1.049553	101.3526	0.000000
logdose	32.2245	1.703929	18.9119	0.000000

Két külön egyenes
y vs. logdózis
itt már adekvátak

A két egyenest együtt kezelve háromféle modell: Separate slopes
Homogeneity of slopes
Analysis of covariance

$$y_{ijk} = \alpha_i + \beta_i x_{ij} + \varepsilon_{ijk}$$

$$y_{ijk} = \mu + \alpha_i + (\alpha\beta)_i x_{ij} + \varepsilon_{ijk}$$

Statistics>Advanced Linear/Nonlinear Models>
>General Linear Models>Separate slopes

Univariate Tests of Significance for y (Wardlawp231.sta)
Over-parameterized model
Type III decomposition; Std. Error of Estimate: 2.408318

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	102589.4	1	102589.35	17687.82	0.000000
prepn*logdose	3602.3	2	1801.13	310.54	0.000000
prepn	86.4	1	86.40	14.90	0.000977
Error	116.0	20	5.80		

β (H_0: vízszintes egyenesek)

α (H_0: nincs kbs. a tengelymetszetben)

Test of Lack of Fit (Wardlawp231.sta)

Dependent Variable	SS Pure Err	df Pure Err	MS Pure Err	SS Lack of Fit	df Lack of Fit	MS Lack of Fit	F	p
y	112.2500	18	6.236111	3.750000	2	1.875000	0.300668	0.743970

$$y_{ijk} = \mu + \alpha_i + (\alpha\beta)_i x_{ij} + \varepsilon_{ijk}$$

Effect	Level of Effect	Column	Comment (B/Z/P)	y Param.	y Std.Err
	Parameter Estimates (Wardlawp231.sta) (*Zeroed predictors failed tolerance check) Over-parameterized model				
Intercept		1		100.3750	1.099242
prepn*logdose	1	2		32.2245	1.784597
prepn*logdose	2	3		30.6526	1.784597
prepn	standard	4	Biased	6.0000	1.554563
prepn	sample	5	Zeroed*	0.0000	

μ
$(\alpha\beta)_1$
$(\alpha\beta)_2$
α_1
α_2

ugyanazok a becsült paraméterek, mint amikor külön illesztettük az egyeneseket

Label	Column	Variable	Level of Variable	Variable	Level of Variable
	Column Labels (Wardlawp231.sta) Labels for the columns of the design matrix X				
Intercept	1				
prepn*logdose	2	prepn	standard	logdose	
prepn*logdose	3	prepn	sample	logdose	
prepn	4	prepn	standard		
prepn	5	prepn	sample		

$$y_{ijk} = \mu + \alpha_i + \beta x_{ij} + (\alpha\beta)_i x_{ij} + \varepsilon_{ijk}$$

β a vonatkozási egyenes meredeksége

Statistics>Advanced Linear/Nonlinear Models>
>General Linear Models>
>Homogeneity-of-slopes

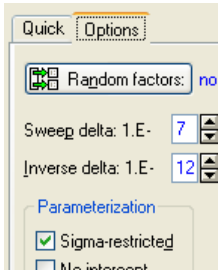
Effect	SS	Degr. of Freedom	MS	F	p
	Univariate Tests of Significance for y Sigma-restricted parameterization Std. Error of Estimate: 2.408318				
Intercept	102589.3	1	102589.3	17687.82	0.000000
prepn	86.4	1	86.4	14.90	0.000977
logdose	3600.0	1	3600.0	620.69	0.000000
prepn*logdose	2.3	1	2.3	0.39	0.540427
Error	116.0	20	5.8		

α (H_0: nincs kbs. a tengelymetszetben)
β (H_0: a közös egyenes vízszintes)
$\alpha\beta$ (H_0: párhuzamos egyenesek)

β a vonatkozási egyenes meredeksége

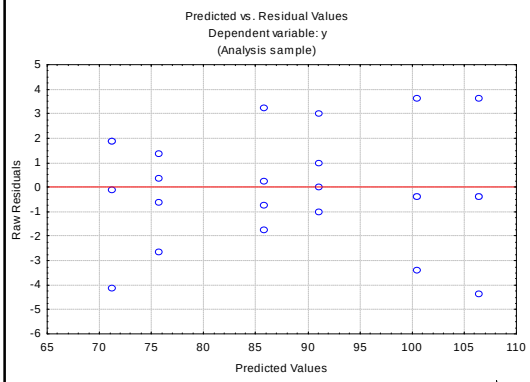
$$y_{ijk} = \mu + \alpha_i + \beta x_{ij} + (\alpha\beta)_i x_{ij} + \varepsilon_{ijk}$$

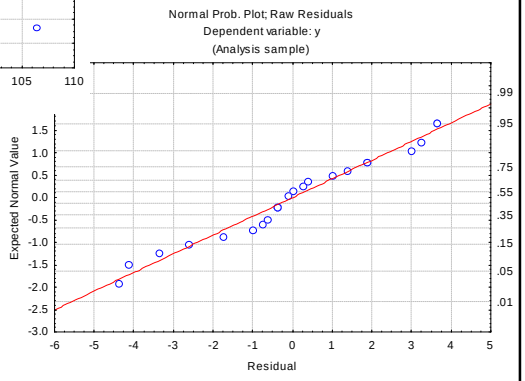
Effect	Level of Effect	Column	y Param.	y Std.Err
	Parameter Estimates (Wardlawp231.sta) Sigma-restricted parameterization			
Intercept		1	103.3750	0.777282
prepn	standard	2	3.0000	0.777282
logdose		3	31.4385	1.261901
prepn*logdose	1	4	0.7860	1.261901



Effect	Level of Effect	Column	Comment (B/Z/P)	y Param.	y Std.Err
	Parameter Estimates (Wardlawp231.sta) (*Zeroed predictors failed tolerance check) Over-parameterized model				
Intercept		1		100.3750	1.099242
prepn	standard	2	Biased	6.0000	1.554563
prepn	sample	3	Zeroed*	0.0000	
logdose		4		30.6526	1.784597
prepn*logdose	1	5	Biased	1.5719	2.523802
prepn*logdose	2	6	Zeroed*	0.0000	

μ
α_1
α_2
β
$(\alpha\beta)_1$
$(\alpha\beta)_2$





Univariate Tests of Significance fory (Wardlawp231.sta)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 2.372962

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	102589.3	1	102589.35	18218.83	0.000000
logdose	3600.0	1	3600.00	639.32	0.000000
prepn	165.4	1	165.38	29.37	0.000022
Error	118.2	21	5.63		

Statistics>Advanced Linear/Nonlinear Models>
>General Linear Models>
>Analysis of Covariance

Parameter Estimates (Wardlawp231.sta)
Sigma-restricted parameterization

Effect	Level of Effect	Column	y Param.	y Std.Err	y t	y p
Intercept		1	103.3750	0.765870	134.9771	0.000000
logdose		2	31.4385	1.243375	25.2848	0.000000
prepn	standard	3	2.6250	0.484379	5.4193	0.000022

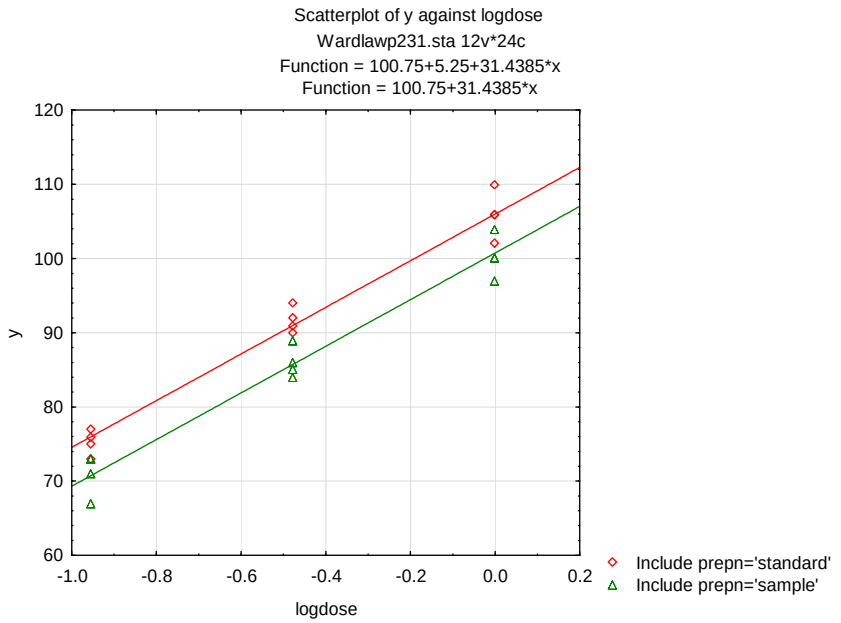
$$y_{ijk} = \mu + \alpha_i + \beta x_{ij} + \varepsilon_{ijk}$$

Parameter Estimates (Wardlawp231.sta)
(*Zeroed predictors failed tolerance check)
Over-parameterized model

Effect	Level of Effect	Column	Comment (B/Z/P)	y Param.	y Std.Err
Intercept		1		100.7500	0.906190
logdose		2		31.4385	1.243375
prepn	standard	3	Biased	5.2500	0.968758
prepn	sample	4	Zeroed*	0.0000	

$$y_{ijk} = \alpha + (\alpha_i - \alpha) + \beta x_{ij} + \varepsilon_{ijk}$$

α a vonatkozási egyenes tengelymetszete



A minta aktivitásának számítása

$$\hat{Y} = a + b\ln c = a + b\lg\frac{c_0}{h} = a + b\lg c_0 - b\lg h = a + b\lg c_0 + b\lg x$$

x a dózis (a h hígítás reciproka), lg$x$ a dózis logaritmusa,

c_0 a készítmény hígítás előtti koncentrációja

Ismert a standard hígítás előtti $c_{0\text{std}}$ koncentrációja (5NE/ml),

kérdés a vizsgálandó készítmény hígítás előtti $c_{0\text{minta}}$ koncentrációja

Az illesztett egyenes:

$\hat{Y} = a' + b\lg x$ a' különböző a készítményekre

Mennyivel nagyobb log dózis kell a mintából, hogy azonos hatást (abszorbanciát) adjon a standarddal:

$\hat{Y}_{\text{minta}} = \hat{Y}_{\text{std}}$ (különböző dózisnál)

$\hat{Y}_{\text{minta}} = \hat{Y}_{\text{std}}$ (különböző dózisnál)

$\hat{Y} = a' + b\lg x$

$$\lg x_{\text{minta}} - \lg x_{\text{std}} = \frac{a'_{\text{minta}} - a'_{\text{std}}}{b} = \frac{-5.25}{31.4385} = -0.1670$$

$$a + b(\lg c_{0\text{minta}} + \lg x_{\text{minta}}) = a + b(\lg c_{0\text{std}} + \lg x_{\text{std}})$$

$$\lg c_{0\text{minta}} - \lg c_{0\text{std}} = \lg x_{\text{std}} - \lg x_{\text{minta}}$$

$$\frac{c_{0\text{minta}}}{c_{0\text{std}}} = 10^{\frac{a'_{\text{minta}} - a'_{std}}{b}} = 10^{\frac{-5.25}{31.4385}} = 0.68$$ relative potency

$$c_{0\text{minta}} = 0.68 c_{0\text{std}} = 0.68 \cdot 5 = 3.4\text{NE/ml}$$

Konfidencia-intervallum $CM'_T \pm \sqrt{(C-1)\left[C(M'_T)^2 + 2V\right]}$

$$C = \frac{S_{regr}}{S_{regr} - t^2_{\alpha/2} s_r^2} \qquad M'_T = \lg x_{\text{minta}} - \lg x_{\text{std}}$$

$$V = \frac{S_{regr}}{b^2 dn}$$ *d* a dózisok száma, *n* az ismétlések száma

$$C = \frac{S_{regr}}{S_{regr} - t^2_{\alpha/2} s_r^2} = \frac{3600}{3600 - 2.1^2 \cdot 6.236} = 1.0077$$

$$V = \frac{S_{regr}}{b^2 dn} = \frac{3600}{31.4385^2 \cdot 3 \cdot 4} = 0.3035$$

$$M'_T = \lg x_{\text{minta}} - \lg x_{\text{std}} = -0.1670$$

$$CM'_T \pm \sqrt{(C-1)\left[C(M'_T)^2 + 2V\right]} =$$

$$= 1.0770 \cdot (-0.1670) \pm \sqrt{0.0770 \cdot \left[1.0770 \cdot (-0.1670)^2 + 2 \cdot 0.3035\right]} =$$

$$= -0.1682 \pm 0.0699 = (-0.238, -0.0984)$$

$$\frac{c_{0\text{minta}}}{c_{0\text{std}}} = (0.578, 0.797)$$

2. példa

Biotechnológiai készítmény titerét kívánták meghatározni az ismert aktivitású nemzetközi standardhoz képest. Az analitikai jel a spektrofotometriás abszorbancia volt.

parall1.sta

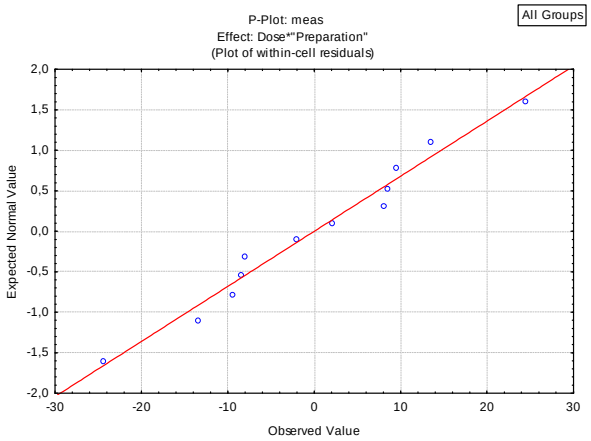
	1 Dose	2 Preparation	3 meas	4 logdose	5 logmeas
1	100	standard	929	2	2.968016
2	100	standard	978	2	2.990339
3	50	standard	636	1.69897	2.803457
4	50	standard	655	1.69897	2.816241
5	25	standard	428	1.39794	2.631444
6	25	standard	445	1.39794	2.64836
7	100	minta	972	2	2.987666
8	100	minta	999	2	2.999565
9	50	minta	638	1.69897	2.804821
10	50	minta	654	1.69897	2.815578
11	25	minta	428	1.39794	2.631444
12	25	minta	424	1.39794	2.627366

Az abszorbancia-adatok igényelnek-e valamilyen transzformációt?

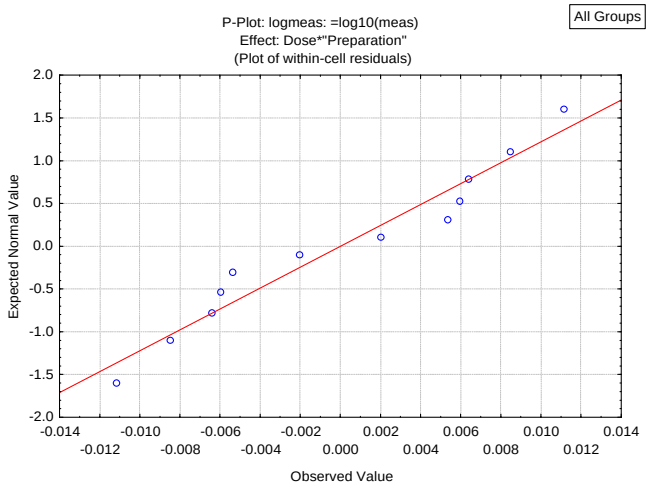
	Tests of Homogeneity of Variances (parall1 Effect: Dose*"Preparation"				
	Hartley F-max	Cochran C	Bartlett Chi-Sqr.	df	p
meas	150.0625	0.592547	3.486854	5	0.625378

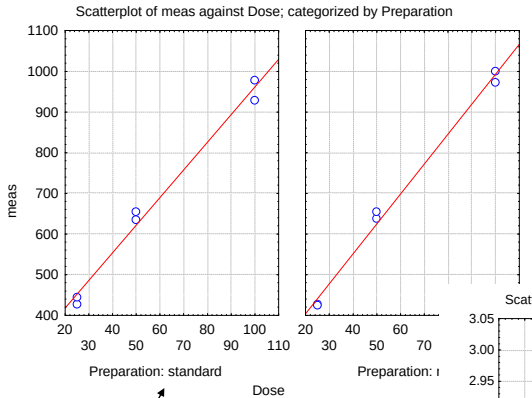
$\sigma_y^2 = konst$ teljesül?

y normális eloszlása teljesül?



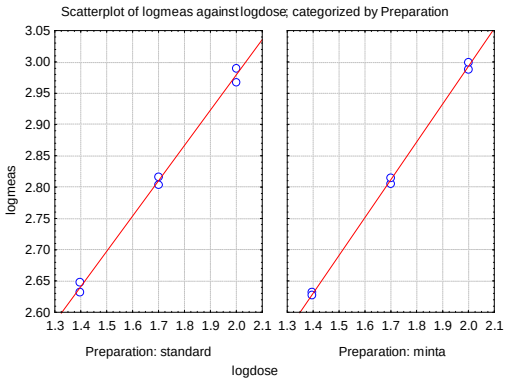
	Tests of Homogeneity of Variances (parall1.st Effect: Dose*"Preparation"				
	Hartley F-max	Cochran C	Bartlett Chi-Sqr.	df	p
logmeas	29.96642	0.407842	1.741484	5	0.883633





Az abszorbanciát ill. a dózist indokolt-e transzformálni a függvény linearitása szempontjából?

így lineáris?



Statistics>Advanced Linear/Nonlinear Models>
>General Linear Models>Separate slopes

$$y_{ijk} = \alpha_i + \beta_i x_{ij} + \varepsilon_{ijk}$$

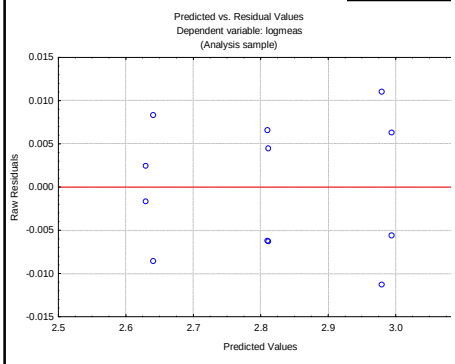
Effect	Univariate Tests of Significance for logmeas (parall1.sta Over-parameterized model Type III decomposition; Std. Error of Estimate: .0087560 SS	Degr. of Freedom	MS	F	p
Intercept	0.812863	1	0.812863	10602.34	0.000000
Preparation*logdose	0.247757	2	0.123879	1615.78	0.000000
Preparation	0.000292	1	0.000292	3.81	0.086663
Error	0.000613	8	0.000077		

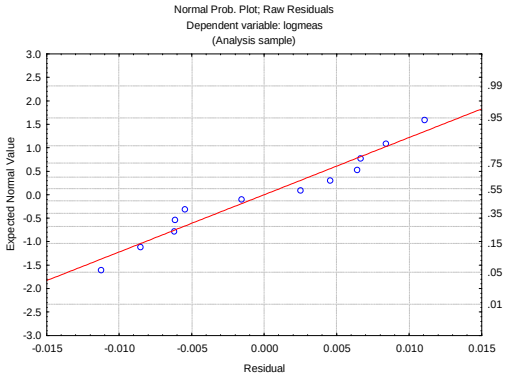
Dependent Variable	Test of Lack of Fit (parall1.sta) SS Pure Err	df Pure Err	MS Pure Err	SS Lack of Fit	df Lack of Fit	MS Lack of Fit	F	p
logmeas	0.000611	6	0.000102	0.000002	2	0.000001	0.011883	0.988210

$$y_{ijk} = \mu + \alpha_i + \beta x_{ij} + (\alpha\beta)_i x_{ij} + \varepsilon_{ijk}$$

Statistics>Advanced Linear/Nonlinear Models>
>General Linear Models>
>Homogeneity-of-slopes

Effect	Univariate Tests of Significance for logmeas Sigma-restricted parameterization Std. Error of Estimate: .0087560 SS	Degr. of Freedom	MS	F	p
Intercept	0.8129	1	0.8129	10602.34	0.00000
Preparation	0.0003	1	0.0003	3.81	0.08666
logdose	0.2474	1	0.2474	3227.50	0.00000
Preparation*logdose	0.0003	1	0.0003	4.06	0.07882
Error	0.0006	8	0.0001		





Effect	Univariate Tests of Significance for logmeas Sigma-restricted parameterization Effective hypothesis decomposition; SS	Degr. of Freedom	MS	F	p
Intercept	0.812863	1	0.812863	7915.4	0.0000
logdose	0.247447	1	0.247447	2409.6	0.0000
Preparation	0.000006	1	0.000006	0.1	0.8123
Error	0.000924	9	0.000103		

Statistics>Advanced Linear/Nonlinear Models> >General Linear Models> >Analysis of Covariance

$$y_{ijk} = \mu + \alpha_i + \beta x_{ij} + \varepsilon_{ijk}$$

Effect	Parameter Estimates (parall1.sta) Sigma-restricted parameterization Level of Effect	Column	logmeas Param.	logmeas Std.Err	logmeas t	logmeas p
Intercept		1	1.817764	0.020431	88.96876	0.000000
logdose		2	0.584233	0.011902	49.08735	0.000000
Preparation	standard	3	-0.000715	0.002925	-0.24450	0.812326

μ a vonatkozási egyenes tengelymetszete

Dependent Variable	Test of Lack of Fit (parall1.sta) SS Pure Err	df Pure Err	MS Pure Err	SS Lack of Fit	df Lack of Fit	MS Lack of Fit	F	p
logmeas	0.000611	6	0.000102	0.000313	3	0.000104	1.025700	0.445230

$$y_{ijk} = \mu + \alpha_i + \beta x_{ij} + \varepsilon_{ijk}$$

Effect	Parameter Estimates (parall1.sta) Sigma-restricted parameterization Level of Effect	Column	logmeas Param.	logmeas Std.Err	logmeas t	logmeas p
Intercept		1	1.817764	0.020431	88.96876	0.000000
logdose		2	0.584233	0.011902	49.08735	0.000000
Preparation	standard	3	-0.000715	0.002925	-0.24450	0.812326

$$y_{ijk} = \alpha + (\alpha_i - \alpha) + \beta x_{ij} + \varepsilon_{ijk}$$

Effect	Parameter Estimates (parall1.sta) (*Zeroed predictors failed tolerance check) Over-parameterized model Level of Effect	Column	Comment (B/Z/P)	logmeas Param.	logmeas Std.Err
Intercept		1		1.818479	0.020640
logdose		2		0.584233	0.011902
Preparation	standard	3	Biased	-0.001431	0.005851
Preparation	minta	4	Zeroed*	0.000000	

A minta aktivitásának számítása (x a dózis, a hígítás reciproka)

$$\hat{Y} = a + b\ln c = a + b\lg\frac{c_0}{h} = a + b\lg c_0 - b\lg h = a + b\lg c_0 + b\lg x$$

Ismert a standard hígítás előtti c_0 koncentrációja, kérdés a vizsgálandó készítmény hígítás előtti c_x koncentrációja

Mennyivel nagyobb log dózis kell a mintából, hogy azonos hatást (abszorbanciát) adjon a standarddal:

$\hat{Y}_{\text{minta}} = \hat{Y}_{\text{std}}$ (különböző dózisnál)

$$\ln c_{0\text{minta}} - \ln c_{0\text{std}} = \frac{a'_{\text{minta}} - a'_{\text{std}}}{b} = \frac{1.8184 - 1.8170}{0.5842} = 0.00245$$

Az aktivitás (relative potency) ennek antilogaritmusa: 1.0056.

3. példa

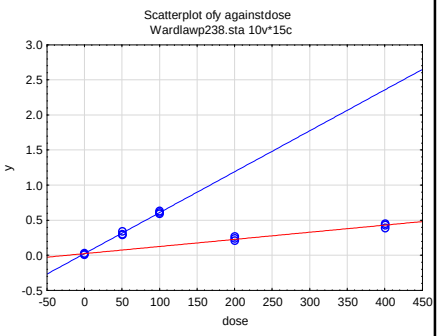
A.C. Wardlaw: Practical statistics for experimental biologists, J. Wiley, 1985, p. 238

Nikotinsav meghatározása szárított barack extraktumban, Lactobacillus plantarum, turbidimetriás eredmények Wardlawp238.sta

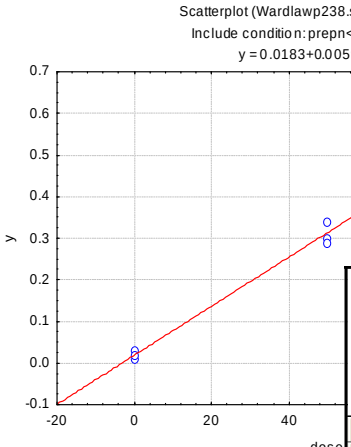
	1 prepn	2 dose	3 rept	4 y
1	standard	50	1	0.3
2	standard	50	2	0.34
3	standard	50	3	0.29
4	standard	100	1	0.59
5	standard	100	2	0.61
6	standard	100	3	0.63
7	sample	200	1	0.27
8	sample	200	2	0.24
9	sample	200	3	0.21
10	sample	400	1	0.43
11	sample	400	2	0.39
12	sample	400	3	0.45
13	blank	0	1	0.03
14	blank	0	2	0.01
15	blank	0	3	0.02

dose: ng ill. ml

Slope ratio assay a szándék



5-pontos



külön a mintára
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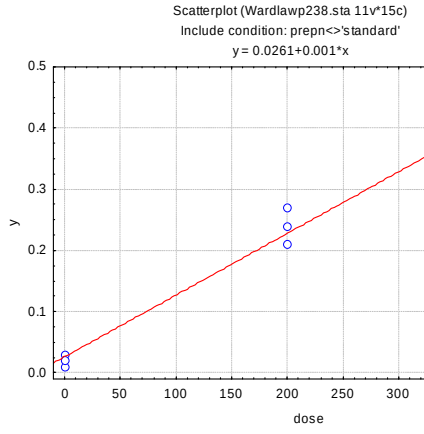
Effect	Univariate Tests of Significance for y (Wardlawp238.sta) Over-parameterized model Type III decomposition Include condition: prepn<>'sample' SS	Degr. of Freedom	MS	F	p
Intercept	0.001210	1	0.001210	3.457	0.105314
dose	0.522150	1	0.522150	1491.857	0.000000
Error	0.002450	7	0.000350		

Dependnt Variable	Test of Lack of Fit (Wardlawp238.sta) Include condition: prepn<>'sample' SS Pure Err	df Pure Err	MS Pure Err	SS Lack of Fit	df Lack of Fit	MS Lack of Fit	F	p
y	0.002400	6	0.000400	0.000050	1	0.000050	0.125000	0.735765



külön a standardre
(+ blank)

Effect	Parameter Estimates (Wardlawp238.sta) Over-parameterized model Include condition: prepn<>'standard' y Param.	y Std.Err	y t	y p
Intercept	0.026111	0.013421	1.94559	0.092768
dose	0.001008	0.000052	19.39921	0.000000

Dependnt Variable	Test of Lack of Fit (Wardlawp238.sta) Include condition: prepn<>'standard' SS Pure Err	df Pure Err	MS Pure Err	SS Lack of Fit	df Lack of Fit	MS Lack of Fit	F	p
y	0.003867	6	0.000644	0.000672	1	0.000672	1.043103	0.346502

	1 prepn	2 dose	3 rept	4 y	5 dosestd	6 dosesampl
1	standard	50	1	0.3	50	0
2	standard	50	2	0.34	50	0
3	standard	50	3	0.29	50	0
4	standard	100	1	0.59	100	0
5	standard	100	2	0.61	100	0
6	standard	100	3	0.63	100	0
7	sample	200	1	0.27	0	200
8	sample	200	2	0.24	0	200
9	sample	200	3	0.21	0	200
10	sample	400	1	0.43	0	400
11	sample	400	2	0.39	0	400
12	sample	400	3	0.45	0	400
13	blank	0	1	0.03	0	0
14	blank	0	2	0.01	0	0
15	blank	0	3	0.02	0	0

$$\hat{Y} = a + b_{\text{minta}} x_{\text{minta}} \qquad \hat{Y} = a + b_{\text{std}} x_{\text{std}}$$

StatisticsStatistics>Advanced Linear/Nonlinear Models>
>General Linear Models>Multiple regression

Effect	Univariate Tests of Significance for y (Wardlawp238.sta) Over-parameterized model Type III decomposition SS	Degr. of Freedom	MS	F	p
Intercept	0.002381	1	0.002381	4.1574	0.064106
dosestd	0.558451	1	0.558451	975.1215	0.000000
dosesampl	0.270561	1	0.270561	472.4318	0.000000
Error	0.006872	12	0.000573		

Dependent Variable	Test of Lack of Fit (Wardlawp238.sta) SS Pure Err	df Pure Err	MS Pure Err	SS Lack of Fit	df Lack of Fit	MS Lack of Fit	F	p
y	0.006067	10	0.000607	0.000806	2	0.000403	0.664050	0.536061

$$\hat{Y} = a + b_{\text{std}} x_{\text{std}}$$

közös tengelymetszetű egyenesek

$$\hat{Y} = a + b_{\text{minta}} x_{\text{minta}}$$

Options fülön: No intercept

Effect	Parameter Estimates (Wardlawp238.sta) Over-parameterized model			
	y Param.	y Std.Err	y t	y p
dosestd	0.006120	0.000138	44.42113	0.000000
dosesampl	0.001087	0.000034	31.54965	0.000000

Dependnt Variable	Test of Lack of Fit (Wardlawp238.sta)							
	SS Pure Err	df Pure Err	MS Pure Err	SS Lack of Fit	df Lack of Fit	MS Lack of Fit	F	p
y	0.006067	10	0.000607	0.003187	3	0.001062	1.750916	0.219806

$$\hat{Y} = b_{std} x_{std}$$

zérus tengelymetszetű egyenesek

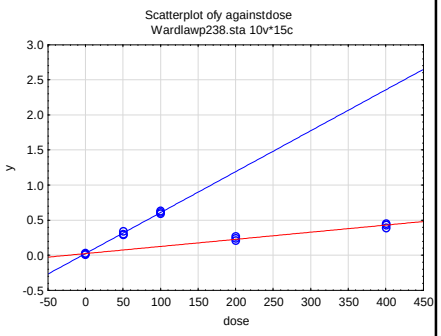
$$\hat{Y} = b_{minta} x_{minta}$$

Bármelyiket használjuk is

$\hat{Y}_{std} = \hat{Y}_{minta}$ helyen $\quad b_{minta} x_{minta} = b_{std} x_{std}$

Effect	Parameter Estimates (Wardlawp238.sta) Sigma-restricted parameterization			
	y Param.	y Std.Err	y t	y p
Intercept	0.023810	0.011677	2.03898	0.064106
dosestd	0.005834	0.000187	31.22694	0.000000
dosesampl	0.001015	0.000047	21.73550	0.000000

maradjon meg a tengelymetszet



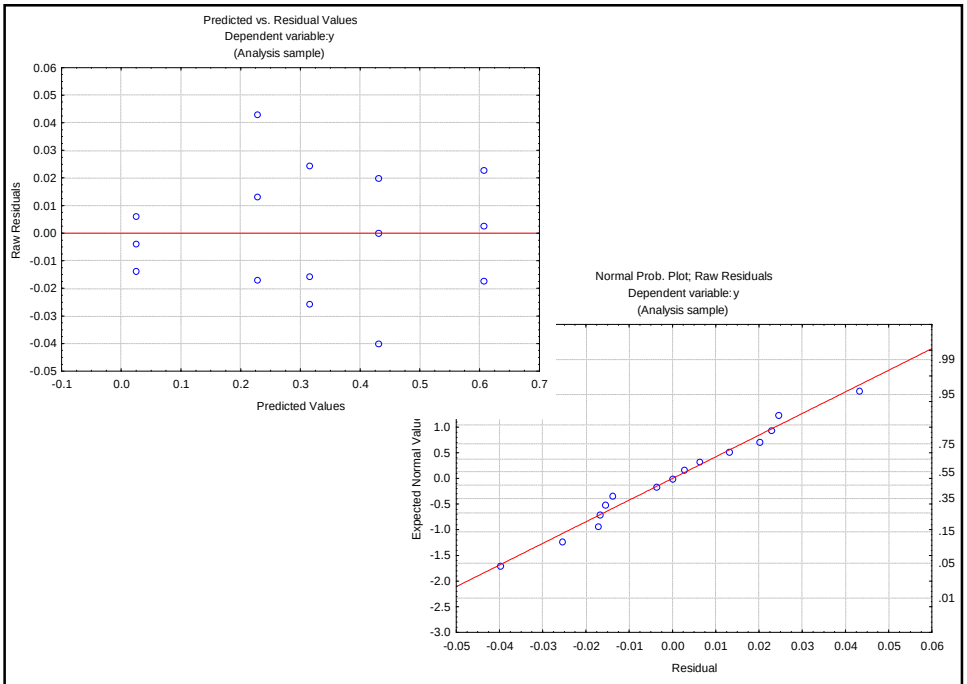
$\hat{Y}_{std} = \hat{Y}_{minta}$ helyen

$$b_{minta} x_{minta} = b_{std} x_{std}$$

$$\frac{x_{minta}}{x_{std}} = \frac{b_{std}}{b_{minta}} = \frac{0.005834}{0.001015} = 5.75$$

$$c_{minta} = \frac{1}{5.75} = 0.174\,\text{ng}/\mu\text{l} = 174\,\text{ng}/\text{ml}$$

vagyis a minta 1ml-e 174ng standardnek felel meg.

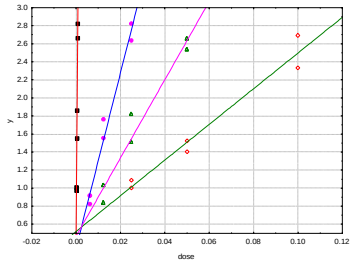


4. példa

3 készítmény standardhoz viszonyított titerét kívánták meghatározni. Az analitikai jel a spektrofotometriás abszorbancia volt. parall2.sta

	1 Prepn	2 Dilut	3 dose	4 lndose	5 Absorb
1	1	10	0.1	-2.30259	2.691
2	1	10	0.1	-2.30259	2.334
3	1	20	0.05	-2.99573	1.524
4	1	20	0.05	-2.99573	1.402
5	1	40	0.025	-3.68888	1.089
6	1	40	0.025	-3.68888	1.001
7	2	20	0.05	-2.99573	2.536
8	2	20	0.05	-2.99573	2.659
9	2	40	0.025	-3.68888	1.513
10	2	40	0.025	-3.68888	1.819
11	2	80	0.0125	-4.38203	1.03
12	2	80	0.0125	-4.38203	0.837
13	3	40	0.025	-3.68888	2.633
14	3	40	0.025	-3.68888	2.819
15	3	80	0.0125	-4.38203	1.551
16	3	80	0.0125	-4.38203	1.759
17	3	160	0.00625	-5.07517	0.82
18	3	160	0.00625	-5.07517	0.918
19	std	1350	0.000741	-7.20786	2.82
20	std	1350	0.000741	-7.20786	2.663
21	std	2700	0.00037	-7.90101	1.863
22	std	2700	0.00037	-7.90101	1.554
23	std	5400	0.000185	-8.59415	1.006
24	std	5400	0.000185	-8.59415	0.976

itt dose=1/hígítás



Slope ratio assay a szándék

	1 Prepn	2 Dilut	3 dose	4 lndose	5 Absorb
1	1	10	0.1	-2.30259	2.691
2	1	10	0.1	-2.30259	2.334
3	1	20	0.05	-2.99573	1.524
4	1	20	0.05	-2.99573	1.402
5	1	40	0.025	-3.68888	1.089
6	1	40	0.025	-3.68888	1.001
7	2	20	0.05	-2.99573	2.536
8	2	20	0.05	-2.99573	2.659
9	2	40	0.025	-3.68888	1.513
10	2	40	0.025	-3.68888	1.819
11	2	80	0.0125	-4.38203	1.03
12	2	80	0.0125	-4.38203	0.837
13	3	40	0.025	-3.68888	2.633
14	3	40	0.025	-3.68888	2.819
15	3	80	0.0125	-4.38203	1.551
16	3	80	0.0125	-4.38203	1.759
17	3	160	0.00625	-5.07517	0.82
18	3	160	0.00625	-5.07517	0.918
19	std	1350	0.000741	-7.20786	2.82
20	std	1350	0.000741	-7.20786	2.663
21	std	2700	0.00037	-7.90101	1.863
22	std	2700	0.00037	-7.90101	1.554
23	std	5400	0.000185	-8.59415	1.006
24	std	5400	0.000185	-8.59415	0.976

Statistics>Advanced Linear/Nonlinear Models>
>General Linear Models>Separate slopes $y_{ijk} = \alpha_i + \beta_i x_{ij} + \varepsilon_{ijk}$

majd nem ezt akarjuk, csak a linearitás vizsgálata kedvéért,

itt még különböző tengelymetszetet is megengedjük

Effect	Parameter Estimates (parall2.sta) (*Zeroed predictors failed tolerance check) Over-parameterized model								
	Level of Effect	Column	Comment (B/Z/P)	Absorb Param.	Absorb Std.Err	Absorb t	Absorb p	-95.00% Cnf.Lmt	+95.00% Cnf.Lmt
Intercept		1		0.475	0.1296	3.661	0.0021	0.200	0.749
Prepn*dose	1	2		19.770	1.9596	10.089	0.0000	15.616	23.924
Prepn*dose	2	3		43.357	3.9192	11.063	0.0000	35.049	51.666
Prepn*dose	3	4		97.131	7.8385	12.392	0.0000	80.515	113.748
Prepn*dose	4	5		3099.214	264.5479	11.715	0.0000	2538.398	3660.031
Prepn	1	6	Biased	0.046	0.1833	0.250	0.8061	-0.343	0.434
Prepn	2	7	Biased	-0.007	0.1833	-0.037	0.9711	-0.395	0.382
Prepn	3	8	Biased	-0.141	0.1833	-0.769	0.4530	-0.530	0.248
Prepn	std	9	Zeroed*	0.000					

Dependnt Variable	Test of Lack of Fit (parall2.sta) SS Pure Err	df Pure Err	MS Pure Err	SS Lack of Fit	df Lack of Fit	MS Lack of Fit	F	p
Absorb	0.252293	12	0.021024	0.106116	4	0.026529	1.261817	0.337791

rendben van a linearitás

	1 Prepn	2 Dilut	3 dose	4 lndose	5 Absorb	6 ve1	7 ve2	8 ve3	9 ve4	10 ve1d	11 ve2d	12 ve3d	13 ve4d
1	1	10	0.1	-2.30259	2.691	1	0	0	0	0.1	0	0	0
2	1	10	0.1	-2.30259	2.334	1	0	0	0	0.1	0	0	0
3	1	20	0.05	-2.99573	1.524	1	0	0	0	0.05	0	0	0
4	1	20	0.05	-2.99573	1.402	1	0	0	0	0.05	0	0	0
5	1	40	0.025	-3.68888	1.089	1	0	0	0	0.025	0	0	0
6	1	40	0.025	-3.68888	1.001	1	0	0	0	0.025	0	0	0
7	2	20	0.05	-2.99573	2.536	0	1	0	0	0	0.05	0	0
8	2	20	0.05	-2.99573	2.659	0	1	0	0	0	0.05	0	0
9	2	40	0.025	-3.68888	1.513	0	1	0	0	0	0.025	0	0
10	2	40	0.025	-3.68888	1.819	0	1	0	0	0	0.025	0	0
11	2	80	0.0125	-4.38203	1.03	0	1	0	0	0	0.0125	0	0
12	2	80	0.0125	-4.38203	0.837	0	1	0	0	0	0.0125	0	0
13	3	40	0.025	-3.68888	2.633	0	0	1	0	0	0	0.025	0
14	3	40	0.025	-3.68888	2.819	0	0	1	0	0	0	0.025	0
15	3	80	0.0125	-4.38203	1.551	0	0	1	0	0	0	0.0125	0
16	3	80	0.0125	-4.38203	1.759	0	0	1	0	0	0	0.0125	0
17	3	160	0.00625	-5.07517	0.82	0	0	1	0	0	0	0.0063	0
18	3	160	0.00625	-5.07517	0.918	0	0	1	0	0	0	0.0063	0
19	std	1350	0.000741	-7.20786	2.82	0	0	0	1	0	0	0	0.000741
20	std	1350	0.000741	-7.20786	2.663	0	0	0	1	0	0	0	0.000741
21	std	2700	0.00037	-7.90101	1.863	0	0	0	1	0	0	0	0.00037
22	std	2700	0.00037	-7.90101	1.554	0	0	0	1	0	0	0	0.00037
23	std	5400	0.000185	-8.59415	1.006	0	0	0	1	0	0	0	0.000185
24	std	5400	0.000185	-8.59415	0.976	0	0	0	1	0	0	0	0.000185

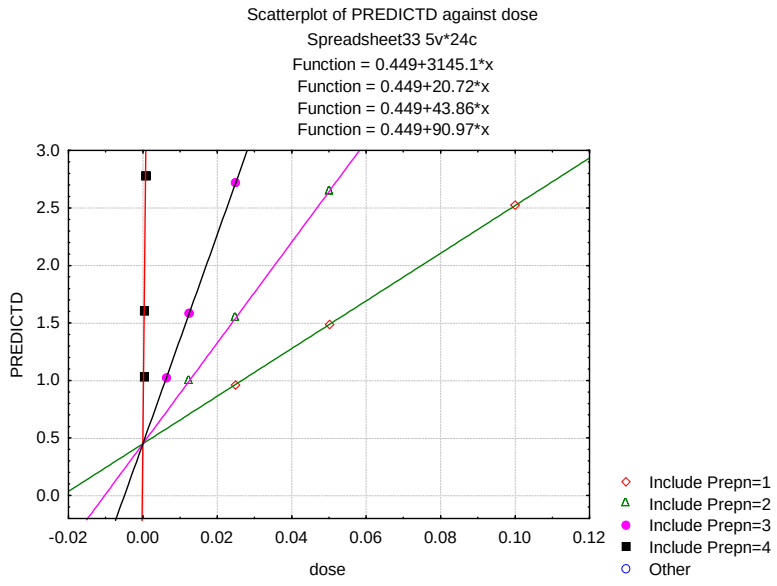
ve1d=ve1*dose…, 4 különböző egyenes

	1 Prepn	2 Dilut	3 dose	4 lndose	5 Absorb	6 ve1d	7 ve2d	8 ve3d	9 ve4d
1	1	10	0.1	-2.30259	2.691	0.1	0	0	0
2	1	10	0.1	-2.30259	2.334	0.1	0	0	0
3	1	20	0.05	-2.99573	1.524	0.05	0	0	0
4	1	20	0.05	-2.99573	1.402	0.05	0	0	0
5	1	40	0.025	-3.68888	1.089	0.025	0	0	0
6	1	40	0.025	-3.68888	1.001	0.025	0	0	0
7	2	20	0.05	-2.99573	2.536	0	0.05	0	0
8	2	20	0.05	-2.99573	2.659	0	0.05	0	0
9	2	40	0.025	-3.68888	1.513	0	0.025	0	0
10	2	40	0.025	-3.68888	1.819	0	0.025	0	0
11	2	80	0.0125	-4.38203	1.03	0	0.0125	0	0
12	2	80	0.0125	-4.38203	0.837	0	0.0125	0	0
13	3	40	0.025	-3.68888	2.633	0	0	0.025	0
14	3	40	0.025	-3.68888	2.819	0	0	0.025	0
15	3	80	0.0125	-4.38203	1.551	0	0	0.0125	0
16	3	80	0.0125	-4.38203	1.759	0	0	0.0125	0
17	3	160	0.00625	-5.07517	0.82	0	0	0.00625	0
18	3	160	0.00625	-5.07517	0.918	0	0	0.00625	0
19	std	1350	0.000741	-7.20786	2.82	0	0	0	0.000741
20	std	1350	0.000741	-7.20786	2.663	0	0	0	0.000741
21	std	2700	0.00037	-7.90101	1.863	0	0	0	0.00037
22	std	2700	0.00037	-7.90101	1.554	0	0	0	0.00037
23	std	5400	0.000185	-8.59415	1.006	0	0	0	0.000185
24	std	5400	0.000185	-8.59415	0.976	0	0	0	0.000185

Statistics>Advanced Linear/Nonlinear Models>
General Linear Models>Multiple regression

Effect	Parameter Estimates (parall2_ve.sta) Sigma-restricted parameterization					
	Absorb Param.	Absorb Std.Err	Absorb t	Absorb p	-95.00% Cnf.Lmt	+95.00% Cnf.Lmt
Intercept	0.449	0.0616	7.29101	0.000001	0.320	0.578
ve1d	20.720	1.2020	17.23835	0.000000	18.204	23.236
"ve2d"	43.857	2.4039	18.24384	0.000000	38.826	48.889
"ve3d"	90.971	4.8079	18.92129	0.000000	80.908	101.034
"ve4d"	3145.114	162.2662	19.38244	0.000000	2805.487	3484.741

közös tengelymetszet



A minta aktivitásának számítása

$$\hat{Y} = a + b_{\text{std}} x_{\text{std}}$$

A nem hígított standard 25NE/ml koncentrációjú, az 1 nagyságú dózis jelentené ugyanezt a koncentrációt, a 0.1-es dózis 2.5NE/ml koncentrációnak felelne meg.

$$\hat{Y} = a + b_{\text{minta}} x_{\text{minta}}$$

Vegyünk a standardból és a készítményből olyan dózisokat, hogy az *y* abszorbancia egyenlő legyen

$$b_{\text{minta}} x_{\text{minta}} = b_{\text{std}} x_{\text{std}} \qquad \frac{x_{\text{std}}}{x_{\text{minta}}} = \frac{b_{\text{minta}}}{b_{\text{std}}}$$

$$\frac{x_{\text{std}}}{x_{\text{minta}}} = \frac{b_{\text{minta}}}{b_{\text{std}}}$$

Pl. a 3. mintára

Effect	Parameter Estimates (parall2_ve.sta) Sigma-restricted parameterization Absorb Param.	Absorb Std.Err	Absorb t	Absorb p	-95.00% Cnf.Lmt	+95.00% Cnf.Lmt
Intercept	0.449	0.0616	7.29101	0.000001	0.320	0.578
ve1d	20.720	1.2020	17.23835	0.000000	18.204	23.236
"ve2d"	43.857	2.4039	18.24384	0.000000	38.826	48.889
"ve3d"	90.971	4.8079	18.92129	0.000000	80.908	101.034
"ve4d"	3145.114	162.2662	19.38244	0.000000	2805.487	3484.741

$$x_{\text{std}} = \frac{b_{\text{minta}}}{b_{\text{std}}} x_{\text{minta}}$$

legyen x_{minta}=1 (hígítatlan)

$$x_{\text{std}} = \frac{b_{\text{minta}}}{b_{\text{std}}} \cdot 1 = \frac{90.97}{3145.11} = 0.029$$

a hígítatlan minta 1/0.029=34.48-szoros hígítású standardnak felel meg