JMP SUMMIT FRANKFURT

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Multivariate Similarity Testing of Dissolution Curves

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Abbott Established Pharmaceuticals

Multivariate Similarity Testing of Dissolution Curves
- agenda

What is dissolution testing and why is it important?

Comparing profiles

Guidance

Approaches

- **fit factor f2**
- **Multivariate analysis**

JMP and multivariate testing

Multivariate Similarity Testing of Dissolution Curves
- what is dissolution testing

Surrogate measure of *in-vivo dissolution*

in-vivo dissolution* rate may affect drug *bio-availability

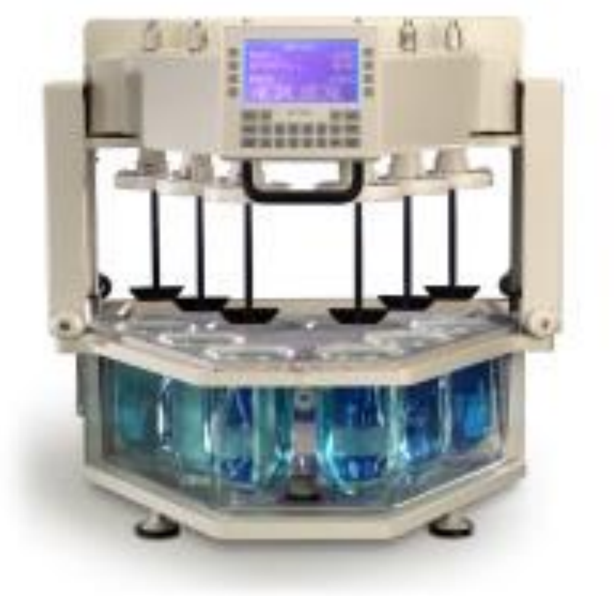
***bio-availability* may affect PK (*blood levels*)**

***blood levels* may affect safety and efficacy**

Compendial requirement for most solid oral dosage forms

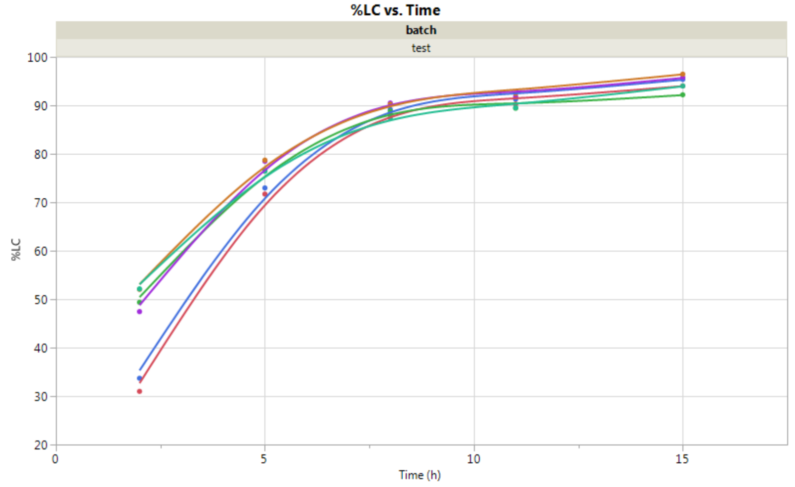
Need to show “equivalence”
for change in product / process / method / site
to obtain a bio-waiver

Multivariate testing of the Similarity of Dissolution Curves
- what is dissolution testing



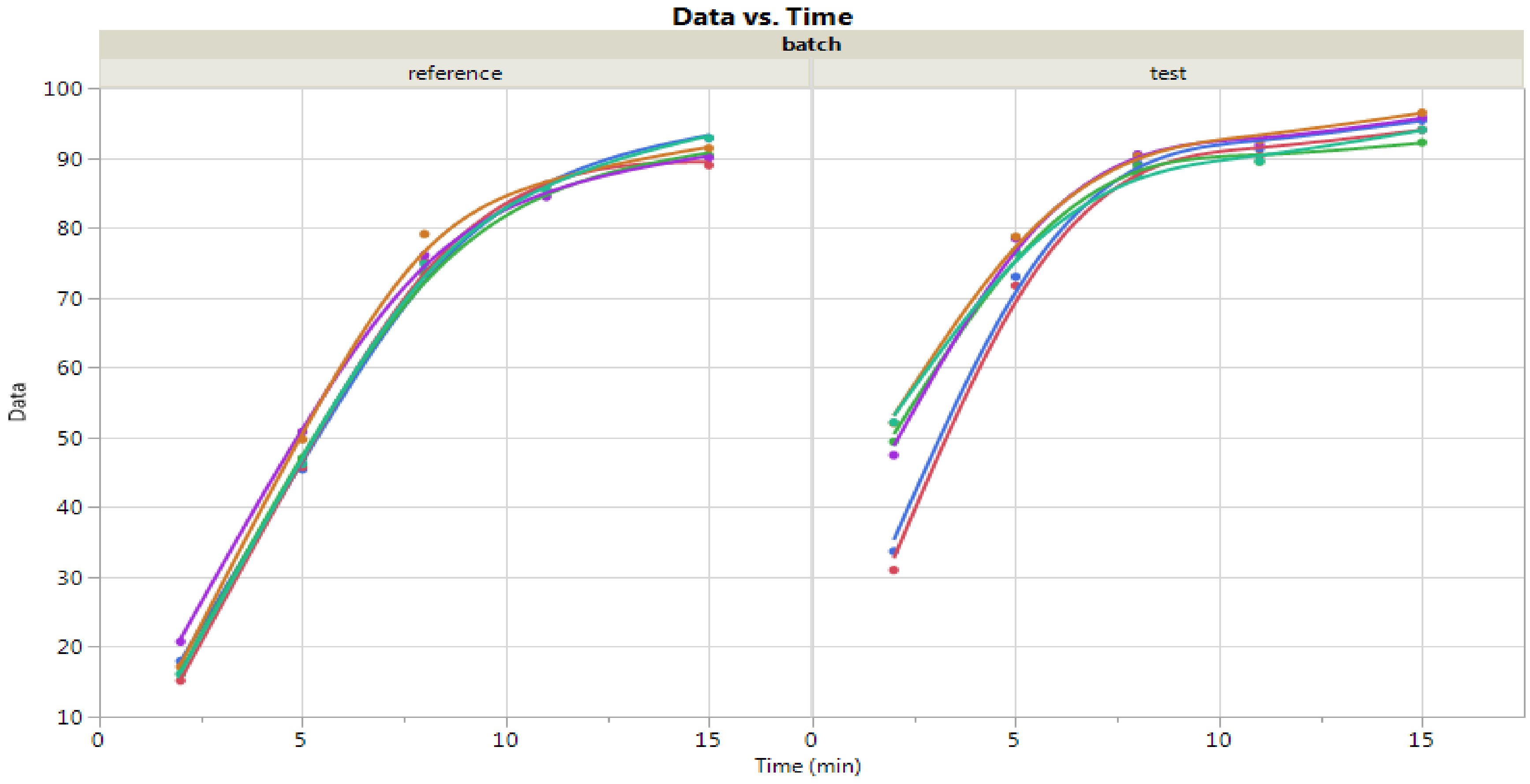
- 1 tablet (unit) / stirred vessel
- each vessel sampled at fixed time intervals
- samples assayed → cumulative concentration
- expressed as % of dosage form Label Content

Time (h)	%LC / unit (tablet)					
	1	2	3	4	5	6
2	34	31	49	47	52	52
5	73	72	77	78	79	76
8	89	88	89	91	90	87
11	91	90	89	92	92	90
15	95	94	92	96	96	94



Multivariate testing of the Similarity of Dissolution Curves
- comparing profiles

Show “equivalence” for product / process / site change to obtain a bio-waiver and avoid lengthy and costly bioavailability study



Similar /
Equivalent profiles?

Multivariate Similarity Testing of Dissolution Curves
- guidance

- guidance is given in papers from the EMA, FDA and other regulatory bodies, which show much resemblance but differ in the details

- similarity is preferably tested using the **f_2** metric, with the following conditions
  - a minimum of 3 time-points (zero excluded)
  - time points should be the same for the two groups (formulations/processes/sites)
  - not more than one mean value >85% dissolved for any of the formulations
  - Coefficient of Variation (CV or RSD) of any product should be
    - less than 20% for the first point(s)
    - less than 10% for subsequent points
  - an f2 value > 50 suggests that the dissolution profiles are similar

- in case the variability is too high, a multivariate statistical distance (**MSD**) metric should be used, either
  - model dependent
  - model independent

Multivariate Similarity Testing of Dissolution Curves
- example

%dissolved		Time point (h)									
		02		05		08		11		15	
batch	tablet	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
reference	1	17.97	.	45.42	.	73.90	.	86.18	.	92.83	.
	2	15.16	.	45.76	.	75.15	.	86.17	.	88.94	.
	3	16.11	.	47.04	.	73.39	.	84.51	.	90.35	.
	4	20.74	.	50.82	.	76.05	.	84.43	.	90.02	.
	5	17.16	.	49.68	.	79.09	.	85.42	.	91.37	.
	6	16.11	.	46.20	.	74.83	.	85.38	.	92.85	.
	All	17.21	11.5	47.49	4.7	75.40	2.7	85.35	0.9	91.06	1.7
test	1	33.69	.	72.97	.	89.18	.	91.28	.	95.36	.
	2	30.99	.	71.71	.	88.12	.	90.27	.	94.03	.
	3	49.37	.	76.61	.	88.82	.	89.42	.	92.20	.
	4	47.45	.	78.48	.	90.50	.	91.83	.	95.77	.
	5	52.05	.	78.71	.	90.24	.	92.47	.	96.46	.
	6	52.14	.	76.43	.	87.40	.	89.55	.	94.01	.
	All	44.28	21.3	75.82	3.8	89.04	1.3	90.80	1.4	94.64	1.6

- exclude 15 h time-point (85% rule)
- 4 time-points left (>3)
- CV > 20% for 1st time-point

→ f_2 metric not allowed

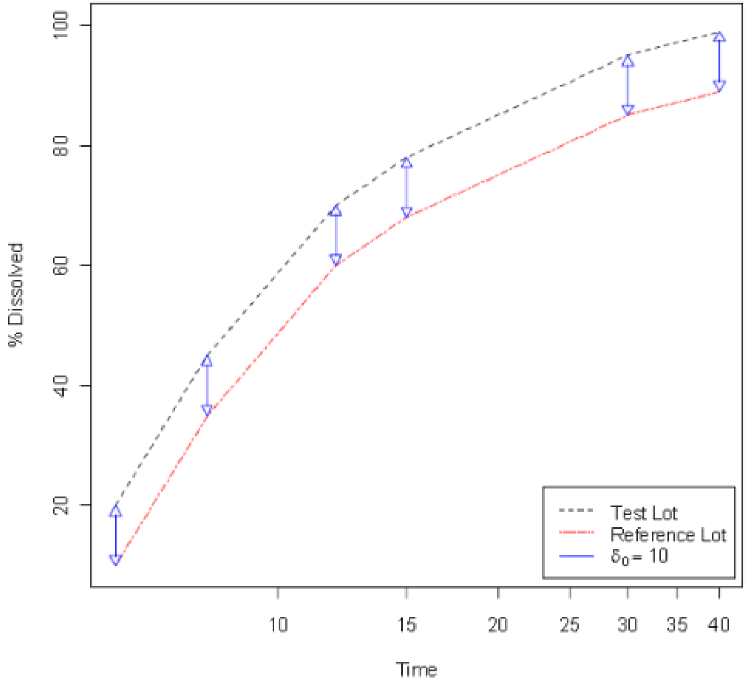
→ use MSD metric

Multivariate Similarity Testing of Dissolution Curves
- f2 metric approach

$$f_2 = 50 \cdot \log\left(\frac{100}{\sqrt{1+\frac{\sum_{t=1}^{n}(R_t - T_t)^2}{n}}}\right) \geq 50$$

$$\sqrt{1+\frac{\sum_{t=1}^{n}(R_t - T_t)^2}{n}} \leq 10$$

- distance estimate = $\hat{f}_2$ (point estimate)
- equivalence: $\hat{f}_2 > 50$ (no measure of uncertainty)



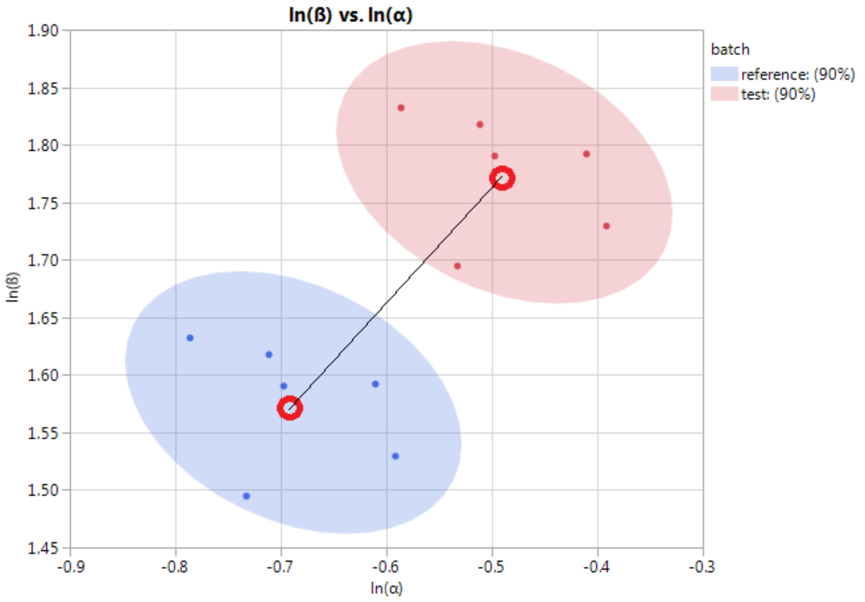
Multivariate Similarity Testing of Dissolution Curves
- Mahalanobis metric approach (MSD)

Two groups of Univariate data

$$d = \frac{\bar{x}_1 - \bar{x}_2}{s_p}$$

Two groups of Multivariate data

$$D^2 = \left(\bar{X}_1 - \bar{X}_2\right)^t \hat{\Sigma}^{-1} \left(\bar{X}_1 - \bar{X}_2\right)$$



$\Sigma =$

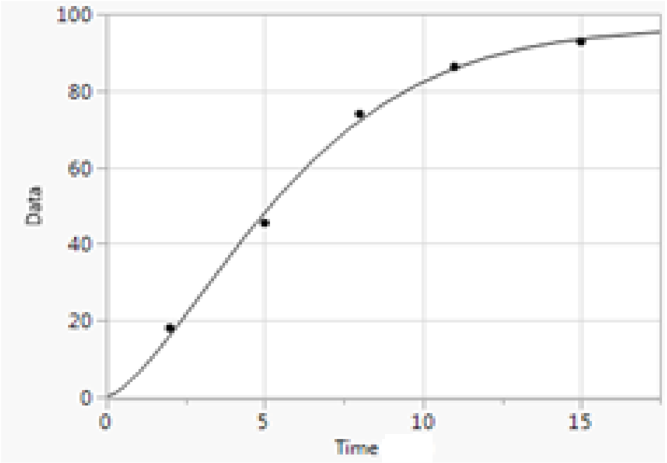
σ_1^2	$\rho_{12}\sigma_1\sigma_2$	$\rho_{13}\sigma_1\sigma_3$	$\rho_{14}\sigma_1\sigma_4$	$\rho_{15}\sigma_1\sigma_5$
$\rho_{12}\sigma_1\sigma_2$	σ_2^2	$\rho_{23}\sigma_2\sigma_3$	$\rho_{24}\sigma_2\sigma_4$	$\rho_{25}\sigma_2\sigma_5$
$\rho_{13}\sigma_1\sigma_3$	$\rho_{23}\sigma_2\sigma_3$	σ_3^2	$\rho_{34}\sigma_3\sigma_4$	$\rho_{35}\sigma_3\sigma_5$
$\rho_{14}\sigma_1\sigma_4$	$\rho_{24}\sigma_2\sigma_4$	$\rho_{34}\sigma_3\sigma_4$	σ_4^2	$\rho_{45}\sigma_4\sigma_5$
$\rho_{15}\sigma_1\sigma_5$	$\rho_{25}\sigma_2\sigma_5$	$\rho_{35}\sigma_3\sigma_5$	$\rho_{45}\sigma_4\sigma_5$	σ_5^2

Multivariate Similarity Testing of Dissolution Curves
- Mahalanobis metric approach (MSD)

Mahalanobis distance

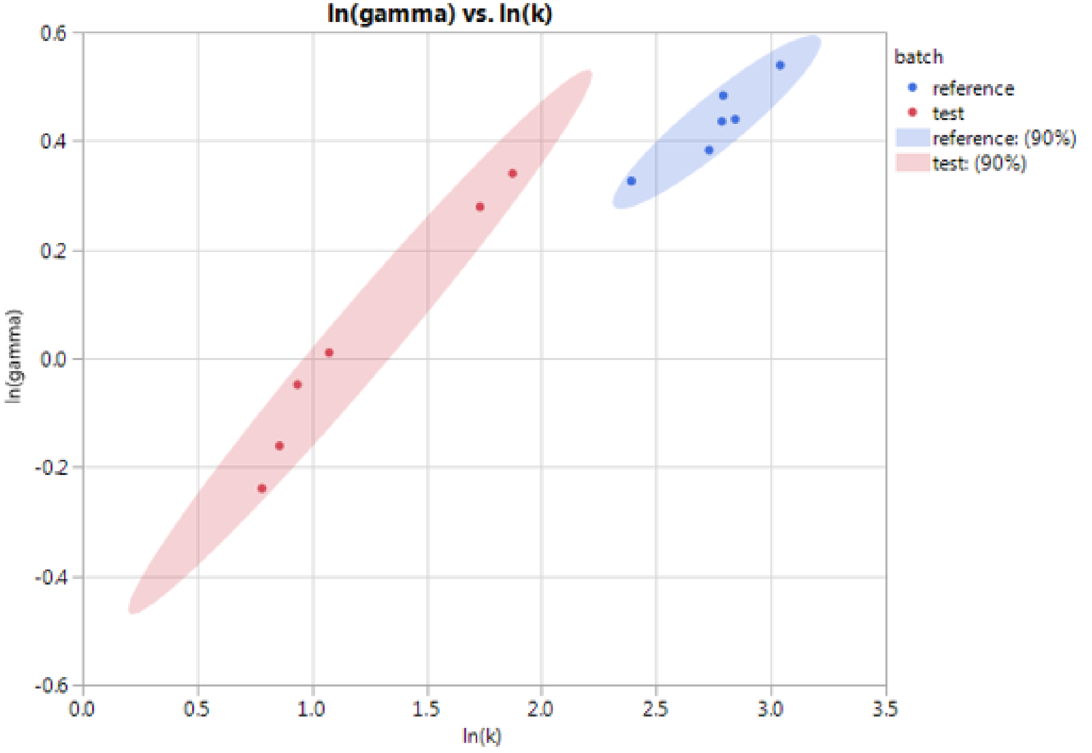
- not based on a (transformed) Euclidian distance
- depends on covariance structure of the data
- can be computed directly on the data (*model free approach*)
- can be computed on the parameters of a model fit to the data (*model based approach*)
  - for each unit (tablet) a pre-defined (non-linear) function is fit, e.g. a Weibull model
  - for the fitted parameters, the MSD is calculated

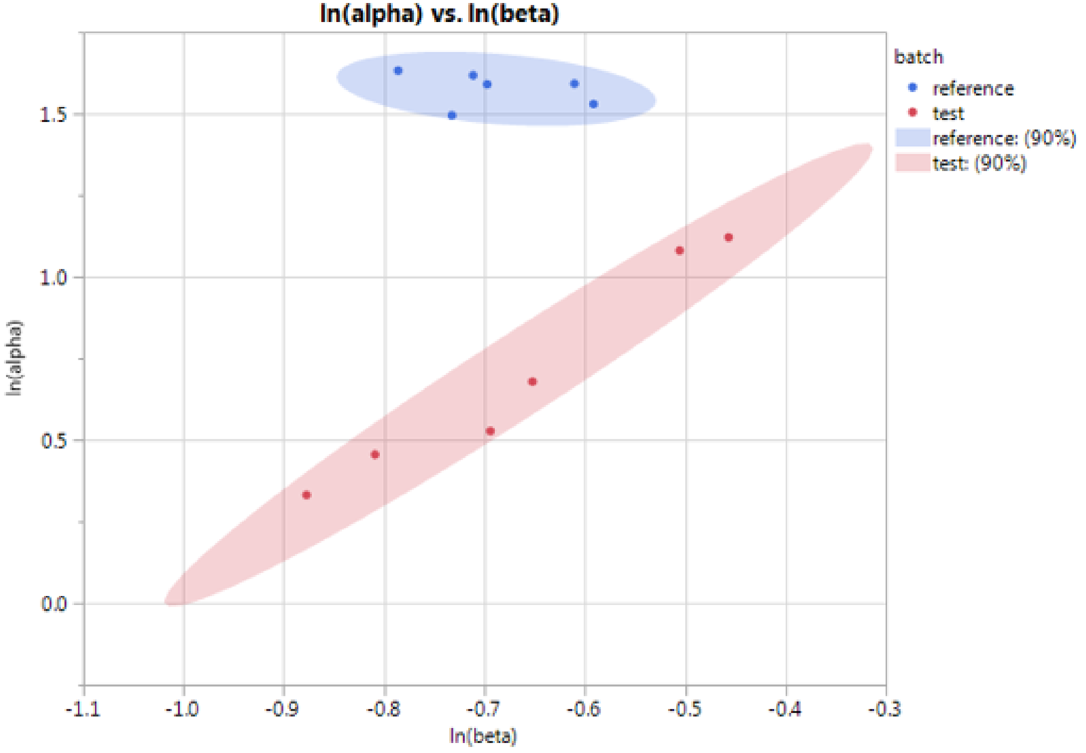
$$g(t) = G_{\max} \times \left(1 - e^{-\frac{1}{\alpha} t^{\beta}}\right)$$



Multivariate Similarity Testing of Dissolution Curves
- Mahalanobis metric approach (MSD)

logistic	Weibull
$f(t) = F_{\max} \times \frac{1}{1+e^{-k(t-\gamma)}}$	$g(t) = G_{\max} \times \left(1 - e^{-\frac{1}{\alpha}t^{\beta}}\right)$





Multivariate Similarity Testing of Dissolution Curves
- Mahalanobis metric approach (MSD)

For a *model free approach*, acceptance limits can be formulated, based on

$$\frac{(n_1+n_2-p-1)}{(n_1+n_2-2)} \cdot \frac{n_1 n_2}{(n_1+n_2)} \cdot D^2 = \frac{(n_1+n_2-p-1)}{(n_1+n_2-2)} \cdot T^2 \approx F_{p,n_1+n_2-p-1}(\lambda)$$

where D^2 represents the Mahalanobis distance, T^2 Hotelling's statistic
and where one could correct for the non-centrality parameter λ, or not (i.e. $\lambda = 0$).

Using the confidence interval, one could decide on statistical significance, but we need to decide on practical relevant differences. Therefore, the following is proposed :

$\lambda = 0$ The acceptance limit is D_{mc} computed as the Mahalanobis distance for a parallel shift of 10%.
Similarity is concluded ⇔ 90%UCL(D_m) < D_{mc}

$\lambda > 0$ A critical distance **d** is calculated
based on simulations, **d** must be less than d_o = 6%

Multivariate Similarity Testing of Dissolution Curves
- Mahalanobis metric approach (MSD) and JMP

Standard functionality

- Graph builder
- Outlier testing
- Non-linear curve fitting
- MANOVA

Extended functionality

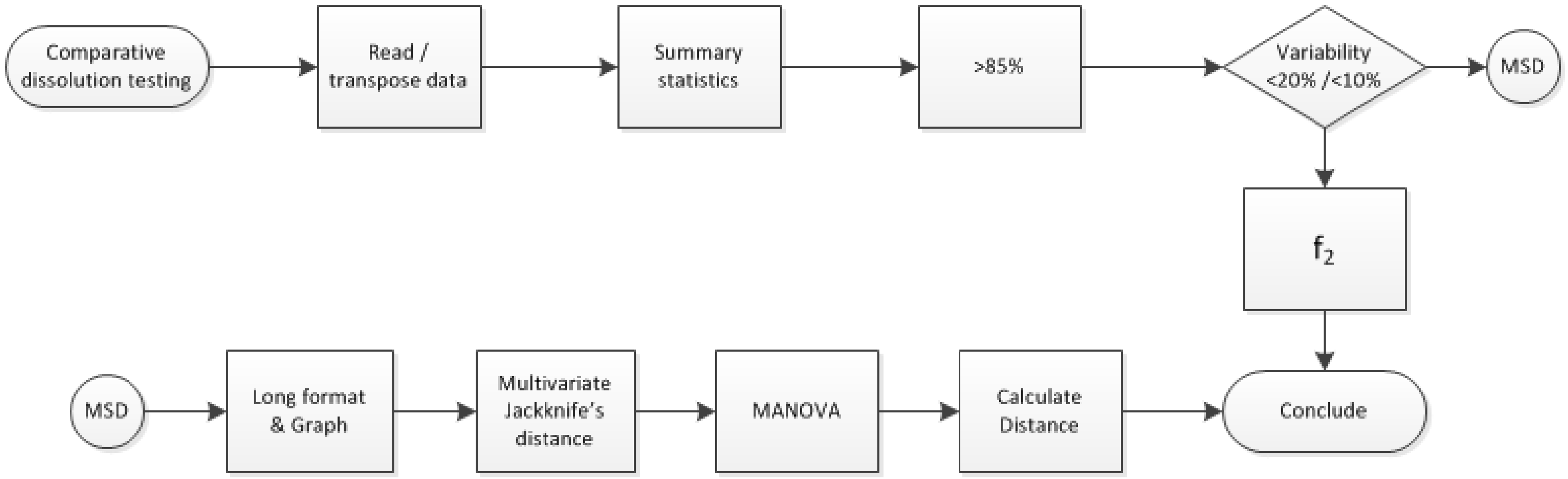
- JSL script
  - Mahalanobis distance
  - Confidence intervals
  - Acceptance limits

Results - JMP

Results of comparison of test to reference.

Quantity	Value
n1	6
n2	6
p	5
k	0.36
Dm	16.0346
Dlower	13.0966
Dupper	18.9727
Dmc	10.5005
d	8.30183
f2	36.2792

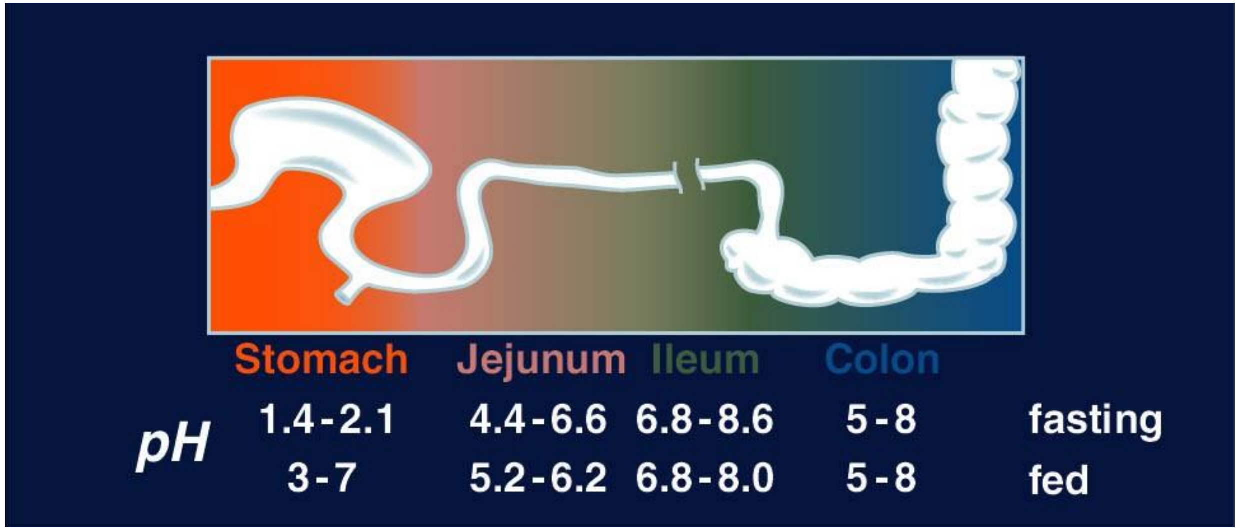
Multivariate Similarity Testing of Dissolution Curves
- Workflow



Multivariate Similarity Testing of Dissolution Curves
- Study design

More extensive studies are performed using

- 3 reference batches
- 3 test batches
- 4 dissolution media
  - pH 1.2
  - pH 4.5
  - pH 6.8
  - QC medium



MODEL FREE APPROACH

JMP demo

Multivariate Similarity Testing of Dissolution Curves
- Study design

Multiple batches complicate the application of the rules for

- just one time point >85%
- CV < 20%

Dissolution summary			t05		t10		t15		t20		t30	
Medium	Type	Batch	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
pH 1.2	Ref	A1	15.9	38.0	37.9	31.0	70.9	16.3	87.8	7.1	96.0	2.6
		A2	9.8	29.7	20.6	18.0	31.8	11.5	46.6	9.7	93.0	4.2
		A3	10.8	41.2	21.8	27.6	35.0	17.3	51.4	15.5	92.2	3.9
	Tst	B1	10.1	28.2	27.2	6.6	48.6	11.2	66.8	9.8	95.0	4.2
		B2	10.2	37.2	22.3	17.8	37.4	19.1	53.8	14.9	93.0	4.3
		B3	11.0	43.9	24.0	35.8	37.8	22.1	50.1	22.8	90.5	5.8
pH 4.5	Ref	A1	32.5	38.1	62.9	27.8	90.7	8.0	96.7	2.5	98.1	1.5
		A2	17.6	22.4	32.4	27.3	50.0	19.5	81.5	11.7	97.3	4.3
		A3	17.3	28.1	36.5	26.9	56.9	23.6	85.9	11.5	96.3	5.3
	Tst	B1	19.2	12.2	39.3	9.9	71.7	13.5	90.4	5.1	95.1	4.8
		B2	12.6	13.3	27.8	17.3	45.2	18.2	71.0	14.2	93.6	4.7
		B3	13.6	28.5	28.7	22.3	43.3	19.4	71.4	15.4	95.7	2.6
pH 6.8	Ref	A1	34.8	24.0	73.8	18.0	96.0	3.9	98.4	2.4	99.2	1.7
		A2	17.3	30.8	29.9	23.7	46.5	27.4	75.1	21.7	97.9	4.5
		A3	28.3	38.6	43.8	30.1	67.0	25.0	87.7	12.3	98.3	3.0
	Tst	B1	18.8	37.7	41.3	28.9	69.3	17.8	88.8	6.6	93.7	6.0
		B2	16.2	30.2	30.8	22.5	47.5	18.9	78.1	10.2	94.3	4.8
		B3	13.7	25.4	27.8	24.4	43.0	25.7	63.3	19.6	92.3	6.5
QC	Ref	A1	14.3	20.7	32.8	20.8	65.0	16.0	87.3	7.1	96.9	2.9
		A2	8.2	20.1	19.2	12.2	30.8	8.6	51.5	18.3	97.8	2.7
		A3	10.5	55.6	24.3	41.9	39.9	28.2	54.5	18.5	95.3	2.7
	Tst	B1	10.8	51.6	27.5	26.7	44.1	18.2	66.3	15.8	93.0	3.1
		B2	13.3	25.7	27.6	25.7	42.8	19.2	58.3	13.0	95.5	3.2
		B3	9.7	31.0	21.8	22.0	35.4	14.1	51.7	12.6	92.8	4.7

f_2 bootstrapping

(and other approaches)

f_2 :   similarity metric, high RSD

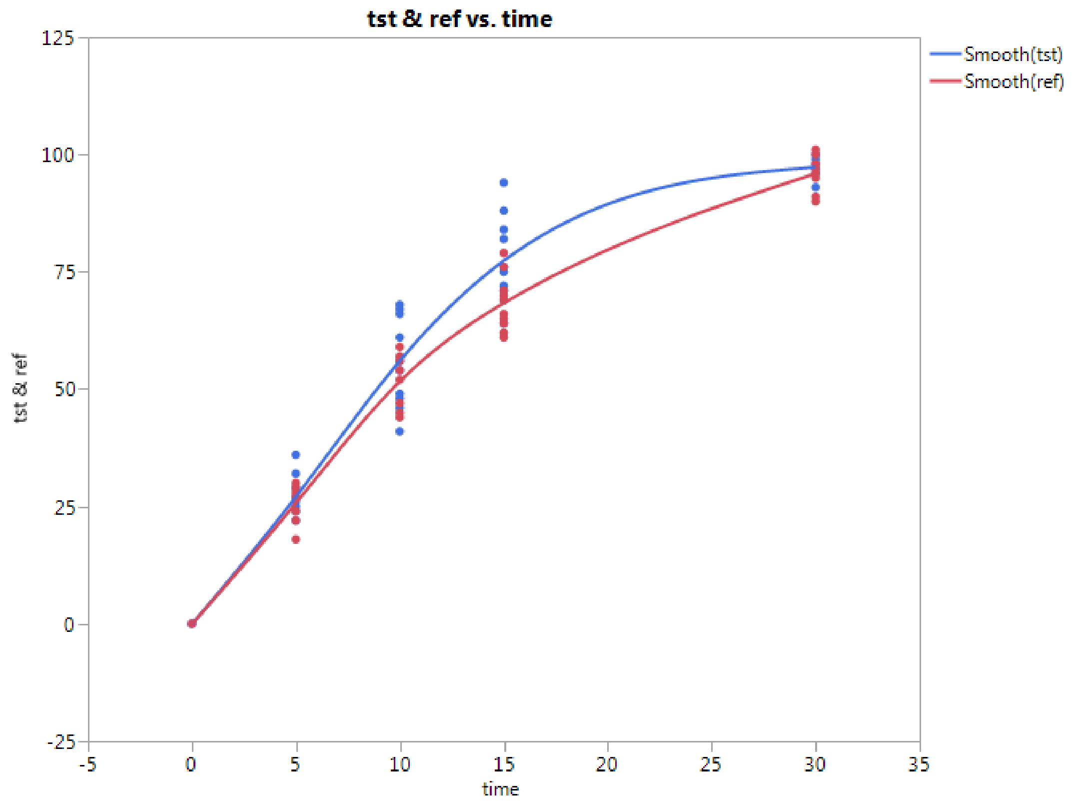
Bootstrapping: an alternative approach

- Problem:
  - no PDF analytically tractable for f_2,
    statistical inference not feasible
- Bootstrapping creates an empirical distribution for f_2
  - Measured data are re-sampled many times (≥ 5000) and for each sample an f_2 value is generated
  - Based on the empirical distribution a 90% LCL is obtained, where LCL > 50, similarity is concluded
- Feasible only when f_2 >> 50
- Implemented using SAS PROC SURVEYSELECT

f_2 : bootstrapping example

tst		5	10	15	30
	1	22	41	64	96
	2	26	54	75	99
	3	36	68	94	97
	4	32	61	76	98
	5	24	49	72	100
	6	24	48	71	98
	7	24	54	82	96
	8	29	67	88	93
	9	29	66	82	96
	10	27	66	84	99
	11	22	46	69	96
	12	25	56	72	99
mean		26.7	56.3	77.4	97.3
RSD		15.8	16.4	11.2	2.0

ref		5	10	15	30
	1	27	59	76	100
	2	24	56	71	101
	3	27	57	69	98
	4	22	44	64	96
	5	29	56	70	96
	6	30	54	69	97
	7	29	52	65	95
	8	18	47	61	91
	9	30	59	79	97
	10	22	45	62	90
	11	28	52	66	95
	12	18	45	66	95
mean		25.3	52.2	68.2	95.9
RSD		17.4	10.7	7.9	3.3



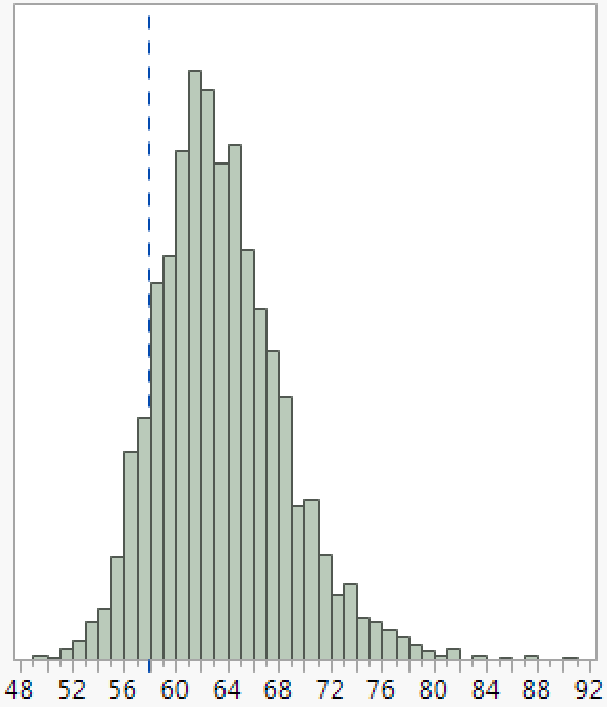
$f_2 = 63.97$

f_2 : bootstrapping example

pdf_example_a - Distribution of F2_ref - JMP

Distributions

F2_ref



Quantiles

100.0%	maximum	90.051499783
99.5%		78.140032079
97.5%		74.214788394
90.0%		69.562901395
75.0%	quartile	66.195437047
50.0%	median	62.981865525
25.0%	quartile	60.154008473
10.0%		57.856456535
2.5%		55.446041778
0.5%		53.051326209
0.0%	minimum	49.316259806

Summary Statistics

Mean	63.438513
Std Dev	4.7359376
Std Err Mean	0.0473831
Upper 95% Mean	63.531393
Lower 95% Mean	63.345633
N	9990

f_2 90% LCL = 57.86 and > 50

f_2 : bootstrapping example

Comparison to other approaches for same data

- **Mahalanobis ($\lambda = 0$)** **pass**
- **Mahalonobis ($\lambda > 0$)** **fail**
- **IUT** **pass**
- **TOST(δ)** **pass**
- **Bootstrap(f_2)** **pass**

IUT = intersection union test

TOST = two-one sided t-interval

δ = SK test statistic for parallel shift

Results - J...

Results of comparison of tst to ref.

Quantity	Value
n1	12
n2	12
p	4
k	1.29545
Dm	1.86304
Dlower	0.54039
Dupper	3.1857
Dmc	4.22233
d	12.9299
f2	63.9695

SK Results - J...

SK Results of comparison of tst to ref.

Quantity	Value
f2	63.9695
max IUT	3.65034
tStat	8.15899
max pvalue	5.35e-6
delta hat	-1.5041
standard error	1.40999
TOST lower	-3.9422
TOST upper	0.93396

"It is fair to say that measuring distances is a topic where a certain amount of arbitrariness seems unavoidable."

Bryan F.J. Manly, Multivariate Statistical Methods, A primer, 2nd Ed.

Multivariate Similarity Testing of Dissolution Curves
- Conclusions

- Dissolution testing is an important aspect of pharmaceutical products in development and quality control

- Comparing dissolution curves is used to support changes (product, production, site)

- Multivariate statistical distance based metrics are used when the variability is too high

- Application is hampered by lack of guidance and regulatory acceptance therefore, different tools are needed

- JMP supports comparing dissolution curves by
  - standard functionality
  - extended functionality using dedicated JSL scripts

Thank you

- **Diane Michelson** (SAS)
  wrote a large part of the code, especially for the interface, helping us to make a fast start in JSL scripting

- **Hadley Myers** (JMP)
  motivated us to share this work

- **Wim Oostra** (Abbott, EPD)
  made valuable comments

Abbott

Multivariate Similarity Testing of Dissolution Curves

90% confidence region Mahalanobis distance, for **λ = 0**:

$$k \times \left(\left(y - \left(x_{test} - x_{ref}\right)\right)^t \times S_{pooled}^{-1} \times \left(y - \left(x_{test} - x_{ref}\right)\right)\right) \le F_{p,n_1+n_2-p-1,0.90}$$

where

$$k = \frac{n_1 n_2}{n_1 + n_2} \cdot \frac{n_1 + n_2 - p - 1}{\left(n_1 + n_2 - 2\right) \times p} = \frac{n_1 n_2}{n_1 + n_2} \cdot k_2 = k_1 \cdot k_2$$

$$\Rightarrow \begin{cases} y_1^* = \left(x_{test} - x_{ref}\right)\left\{1 + \sqrt{\dfrac{F_{p,n_1+n_2-p-1,0.90}}{k\left(x_{test} - x_{ref}\right)^t \times S_{pooled}^{-1} \times \left(x_{test} - x_{ref}\right)}}\right\} \\ y_2^* = \left(x_{test} - x_{ref}\right)\left\{1 - \sqrt{\dfrac{F_{p,n_1+n_2-p-1,0.90}}{k\left(x_{test} - x_{ref}\right)^t \times S_{pooled}^{-1} \times \left(x_{test} - x_{ref}\right)}}\right\} \end{cases}$$

$$\Rightarrow \begin{cases} D_M^{upper} = \max\left(\sqrt{\left(y_1^*\right)^t \times S_{pooled}^{-1} \times y_1^*}, \sqrt{\left(y_2^*\right)^t \times S_{pooled}^{-1} \times y_2^*}\right) \\ D_M^{lower} = \min\left(\sqrt{\left(y_1^*\right)^t \times S_{pooled}^{-1} \times y_1^*}, \sqrt{\left(y_2^*\right)^t \times S_{pooled}^{-1} \times y_2^*}\right) \end{cases}$$

Multivariate Similarity Testing of Dissolution Curves

90% confidence region Mahalanobis distance, for **$\lambda > 0$**:

$$y = k \times D^2 = k \times \left(\left(x_{test} - x_{ref} \right)^t \times S_{pooled}^{-1} \times \left(x_{test} - x_{ref} \right) \right) \approx F_{p, n_1 + n_2 - p - 1}(\lambda)$$

(where $\lambda = k_1 \delta^2$ and δ^2 = population mean of the Mahalanobis distance)

now using an iterative root finder for a monotonic function, find λ_o for

$$y - F_{p, n_1 + n_2 - p - 1, 0.90}(\lambda) = 0$$

(JSL function 'F noncentrality')

then the critical distance **d** is obtained from

$$d = \sqrt{\frac{\lambda_0}{k \times sum\left(S_{pooled}^{-1}\right)}}$$