PIET HOOGKAMER

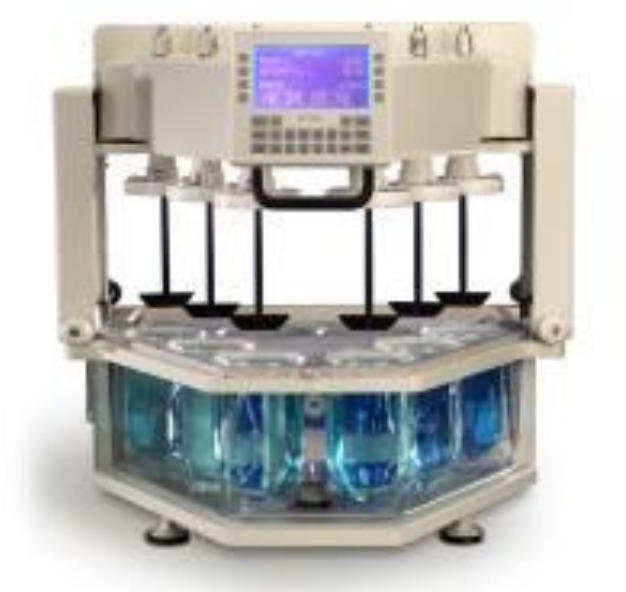
Multivariate Similarity Testing of Dissolution Curves

9 | November | 2023

Agenda

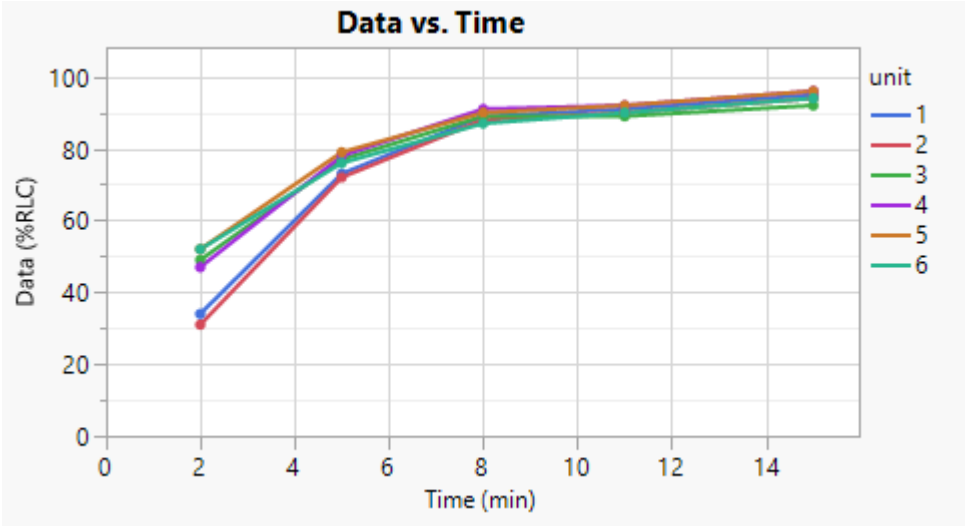
- Abbott Established Pharmaceuticals
- Dissolution testing in vitro vs. in vivo drug administration
- Comparative studies alternative and/or support for biowaiver
- JMP 17 – what is available? curve fitting – model free MVA - bootstrapping
- Regulatory Guidance EMA & AAPS
- JMP 17 – what is missing? E(f2), Hoffelder metrics
- JMP 18 – further support?
- Q&A

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 (%RLC)

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



Dissolution testing

Surrogate measure of *in-vivo dissolution*

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

bio-availability may affect **P**harmaco**K**inetics (*blood levels*)

blood levels may affect safety and efficacy

Compendial requirement for most solid oral dosage forms

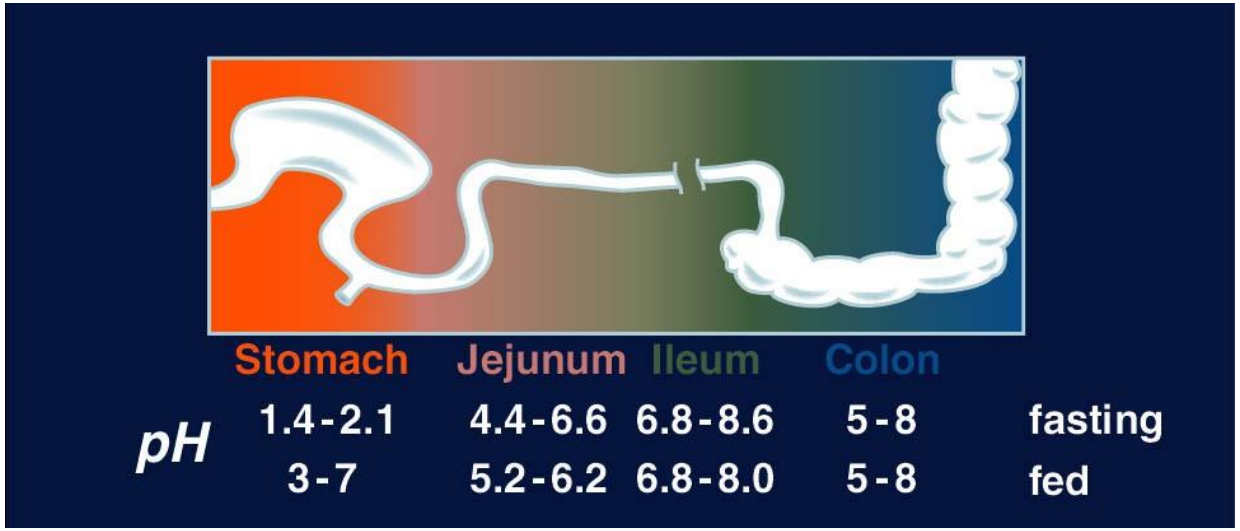
Need to show "similarity" for change in product / process / site

Dissolution profile comparison is an essential tool
in support of *waiving* bioequivalence studies

Comparative dissolution testing

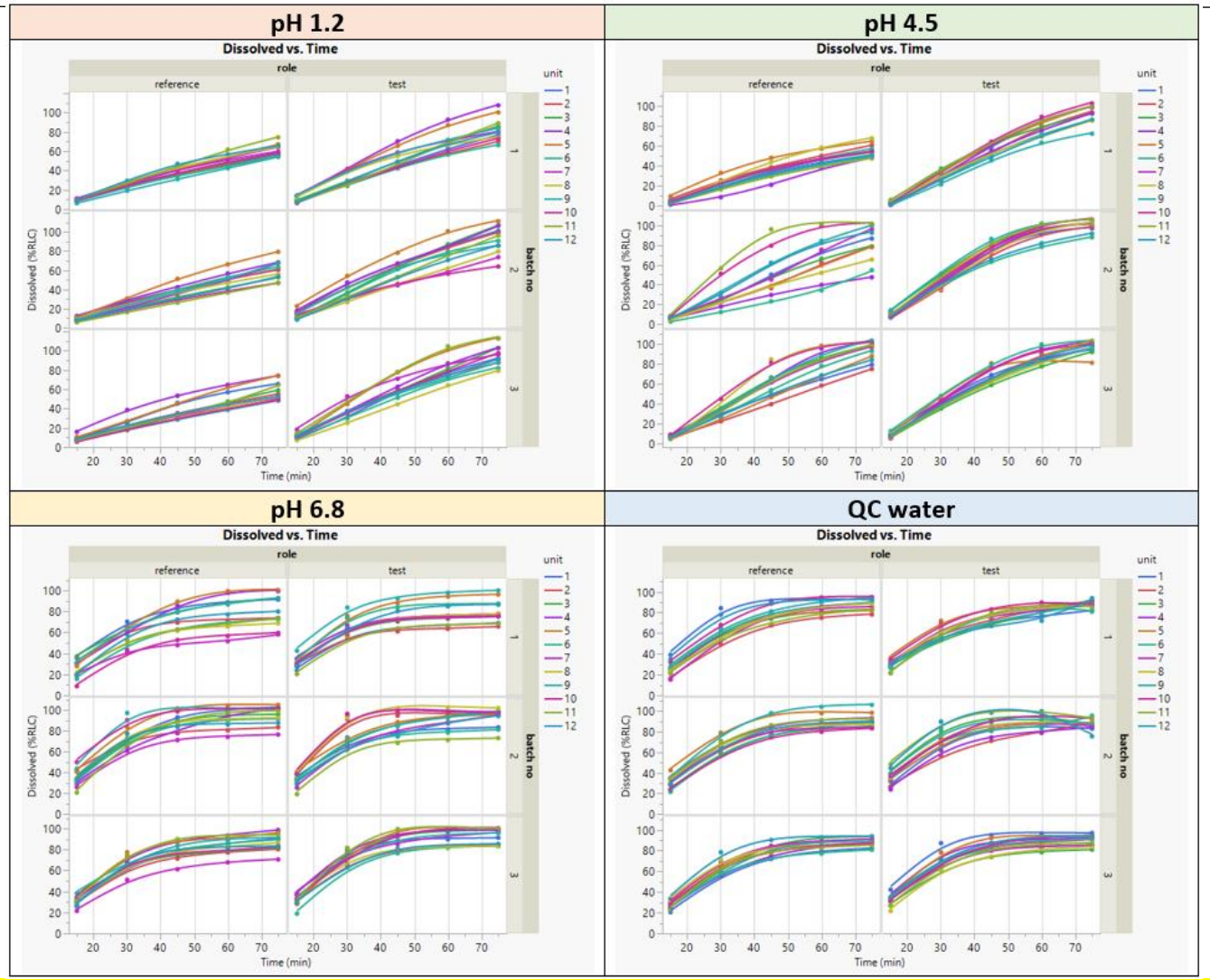
It is more than about comparing one reference batch with one test batch, as typically more extensive studies are performed, using

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



Comparative dissolution testing

Similar /
Equivalent profiles?

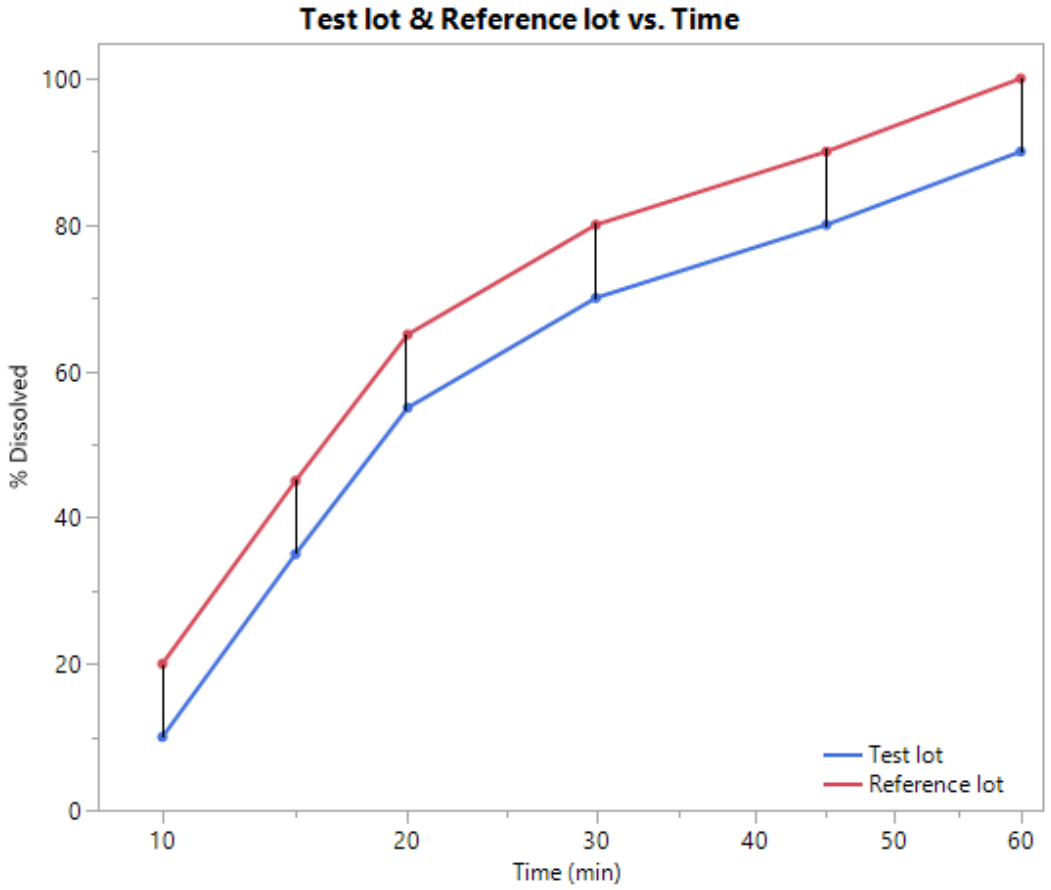


Comparative dissolution testing

$$\hat{f}_2 = 50 \times log\left[\frac{100}{\sqrt{1 + \frac{\sum_{i=1}^{n}(\bar{R}_t - \bar{T}_t)^2}{n}}}\right]$$

- n time-points
- $\bar{R}_t$ *average* dissolution value reference batch
- $\bar{T}_t$ *average* dissolution value test batch

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



Comparative dissolution testing

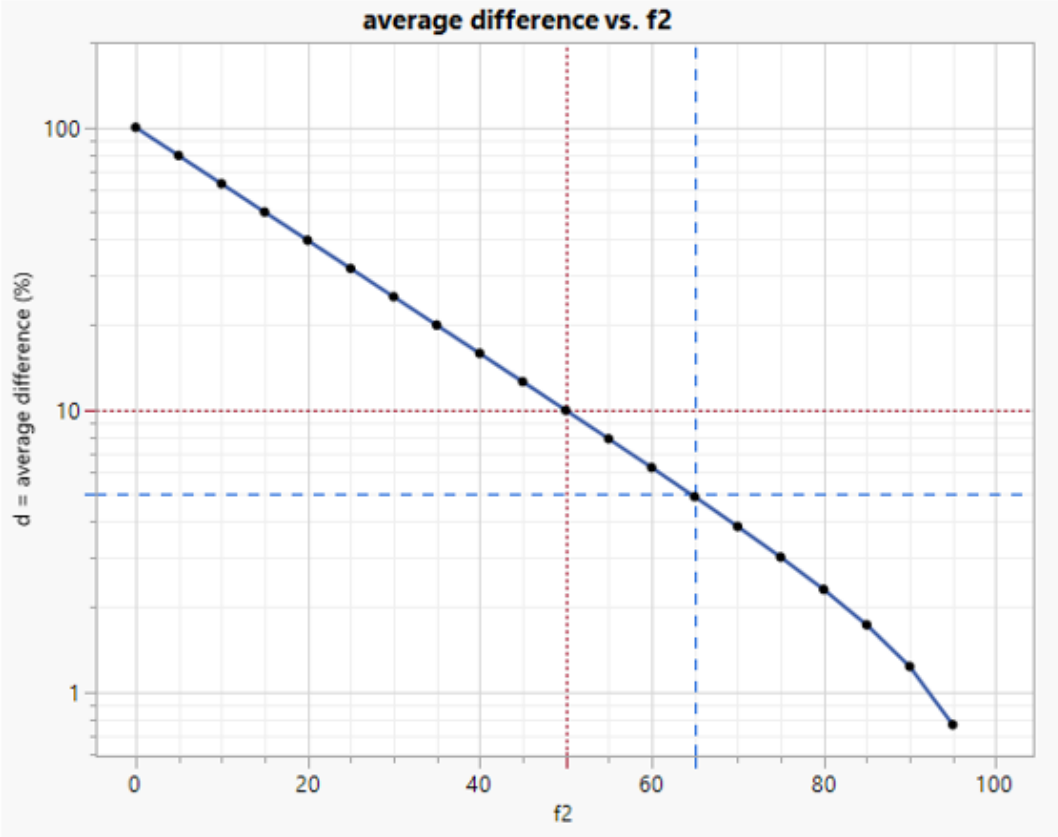
$$\hat{f}_2 = 50 \times log\left[\frac{100}{\sqrt{1 + \frac{\sum_{i=1}^{n}(\bar{R}_t - \bar{T}_t)^2}{n}}}\right]$$

$$\hat{f}_2 = 100 - 25 \times log\left[1 + \frac{\sum_{t=1}^{n}(\bar{R}_t - \bar{T}_t)^2}{n}\right]$$

$$\hat{f}_2 = 100 - 25 \times log[1 + d^2]$$

$$\hat{f}_2 = 50 \sim d = 10$$

i	f_2	d = average difference (%)
1	100	0.0
2	95	0.8
3	90	1.2
4	85	1.7
5	80	2.3
6	75	3.0
7	70	3.9
8	***65***	***4.9***
9	60	6.2
10	55	7.9
11	***50***	***9.9***
12	45	12.5
13	40	15.8
14	35	19.9
15	30	25.1
16	25	31.6
17	20	39.8
18	15	50.1
19	10	63.1
20	5	79.4
21	0	100.0



Comparative dissolution testing - ***rules***

Average values, apply 85% rule

Point	#	1	2	3	4	5	6	7
Time	min	5	10	15	20	30	45	60
Ref	R1	8.28	30.43	56.18	75.51	90.91	96.88	98.67
Ref	R2	10.11	35.88	61.51	80.16	92.58	87.74	99.44
Ref	R3	8.48	29.86	50.64	73.87	91.75	98.48	100.60
Ref	***Rx***	*8.96*	*32.06*	*56.11*	*76.51*	*91.75*	*94.37*	*99.57*
Tst	T1	9.11	33.53	59.62	77.85	91.60	96.91	98.36
Tst	T2	9.41	34.09	58.48	76.27	91.48	97.26	99.17
Tst	T3	10.20	33.99	58.40	76.71	92.35	88.42	99.74
Tst	***Tx***	*9.58*	*33.87*	*58.83*	*76.94*	*91.81*	*94.20*	*99.09*

Coefficient of Variation values (%)

Point	#	1	2	3	4	5	6	7
Time	min	5	10	15	20	30	45	60
Ref	R1	14.08	*10.87*	9.13	3.78	0.78	0.94	1.20
Ref	R2	***27.08***	***16.51***	***11.64***	4.51	1.02	1.08	1.26
Ref	R3	***21.98***	*11.76*	6.48	4.33	1.22	0.75	1.00
Tst	T1	16.04	*11.58*	9.14	4.62	1.59	0.78	0.87
Tst	T2	***24.11***	*14.84*	***11.62***	7.18	1.68	1.41	1.65
Tst	T3	***29.99***	***20.21***	***13.23***	6.84	1.27	1.14	1.12

- exclude 45 and 60 min time-points (85% rule)
- 5 time-points left (≥3)
- CV > 20% for 1st time-point, CV > 10% later points

→ *f_2 metric not allowed*

→ ***use (multivariate) statistical method***

REF	TST	f2
	T1	80
R1	T2	82
	T3	81
	T1	84
R2	T2	79
	T3	80
	T1	66
R3	T2	69
	T3	68
	T1	84
Rx	T2	88
	T3	87

Comparative dissolution testing – Multivariate Statistical Distance

Many approaches have been proposed over the years, most of which are rather complicated:

- based on Mahalanobis Distance
- (M)ANOVA
- non-linear mixed effects models
- principal component analysis
- elaborate modelling
- autoregressive time series
- permutation test
- tolerated difference test
- Bayesian inference
- Bootstrapping
- others …

These approaches often lack a solution to the *calibration* problem:

- Similarity is defined in a statistical sense, but no acceptance criterion is given which is linked to the $f_2 > 50$ rule.

Metrics – multivariate (statistical) distances

- A measure of the distance between two points in multidimensional space is also called a ***metric***
  - The Euclidean Distance (***ED***) is the straight-line distance between two points in ***d*** dimensions

If the coordinates of the positions of P and Q are given by (p_1, p_2, .. , p_d) and (q_1, q_2, .. , q_d), then the Euclidean distance between P and Q is given by:

$$\sqrt{\sum_{j=1}^{d}\left(p_j - q_j\right)^2}$$

The f_2 metric is based on the Euclidean distance, albeit somewhat in disguise, with dimension = time point:

$$f_2 = 100 - 25 \times log\left[1 + \frac{\sum_{j=1}^{d}\left(\bar{R}_j - \bar{T}_j\right)^2}{d}\right]$$

Metrics – multivariate (statistical) distances

- A measure of the distance between two points in multidimensional space is also called a ***metric***
  - The city-block metric in two dimensions measures the distance between two points in a city if, for example, the only directions in which one could travel were north-south and east-west. It is also called the Manhattan distance.

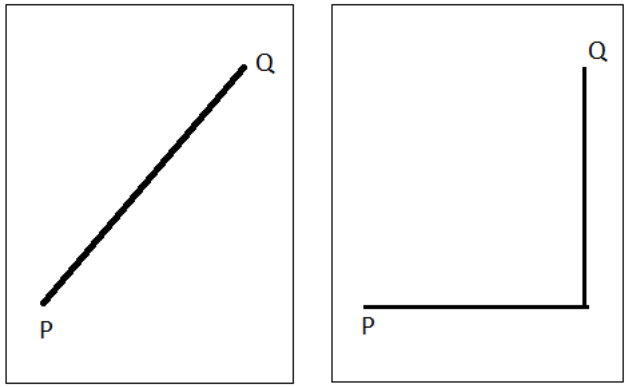
In *d* dimensions the city-block distance between P and Q is:

$$\sum_{j=1}^{d} |p_j - q_j|$$

A generalization is the Minkowski distance:

$$\left\{ \sum_{j=1}^{k} |p_j - q_j|^k \right\}^{\frac{1}{k}}$$

k = 1 Manhattan distance
k = 2 Euclidean distance



Euclidean and city-block metrics, as the simplest of an *infinite* number of possible distance measures.

Metrics – multivariate (statistical) distances

- A measure of the distance between two points in multidimensional space is also called a ***metric***

Some other proposed distance measures are:

- The **Chebyshev distance** is the largest distance over ***d*** dimensions:

$$\max_{1 \le j \le d} |p_j - q_j|$$

- The **Canberra distance** is defined by:

$$\sum_{j=1}^{d} \left\{ \frac{|p_j - q_j|}{|p_j| + |q_j|} \right\}$$

- The **Bray-Curtis distance / Sorensen distance** is given by:

$$\frac{\sum_{j=1}^{d} |p_j - q_j|}{\sum_{j=1}^{d} (p_j + q_j)}$$

Metrics – multivariate (statistical) distances

- While these are all ***mathematical*** distances,
  they do not consider the *variability* per dimension nor the *correlation* along dimensions

A measure that does take the correlations into account is the Mahalanobis distance:

$$MD_{pq}^2 = \sum_{r=1}^{d}\sum_{c=1}^{d}(\mu_{rp} - \mu_{rq})\, v^{rc}\, (\mu_{cp} - \mu_{cq})$$

Where v^{rc} is the element of the rth row and cth column of the inverse of the covariance matrix for the d variables. This can alternatively be written as:

$$MD_{pq}^2 = (\mu_p - \mu_q)'\Sigma^{-1}(\mu_p - \mu_q)$$

where $\mu_i = \begin{bmatrix} \mu_{1i} \\ \mu_{2i} \\ \vdots \\ \mu_{di} \end{bmatrix}$ is the vector of means for population i and $\boldsymbol{\Sigma}$ is the covariance matrix.

Metrics – multivariate (statistical) distances

Covariance matrix **Σ**

$$\Sigma = \begin{pmatrix} \sigma_1^2 & \rho_{12}\sigma_1\sigma_2 & \rho_{13}\sigma_1\sigma_3 & \rho_{14}\sigma_1\sigma_4 & \rho_{15}\sigma_1\sigma_5 & \rho_{16}\sigma_1\sigma_6 \\ \rho_{21}\sigma_2\sigma_1 & \sigma_2^2 & \rho_{23}\sigma_2\sigma_3 & \rho_{24}\sigma_2\sigma_4 & \rho_{25}\sigma_2\sigma_5 & \rho_{26}\sigma_2\sigma_6 \\ \rho_{31}\sigma_3\sigma_1 & \rho_{32}\sigma_3\sigma_2 & \sigma_3^2 & \rho_{34}\sigma_3\sigma_4 & \rho_{35}\sigma_3\sigma_5 & \rho_{36}\sigma_3\sigma_6 \\ \rho_{41}\sigma_4\sigma_1 & \rho_{42}\sigma_4\sigma_2 & \rho_{43}\sigma_4\sigma_3 & \sigma_4^2 & \rho_{45}\sigma_4\sigma_5 & \rho_{46}\sigma_4\sigma_6 \\ \rho_{51}\sigma_5\sigma_1 & \rho_{52}\sigma_5\sigma_2 & \rho_{53}\sigma_5\sigma_3 & \rho_{54}\sigma_5\sigma_4 & \sigma_5^2 & \rho_{56}\sigma_5\sigma_6 \\ \rho_{61}\sigma_6\sigma_1 & \rho_{62}\sigma_6\sigma_2 & \rho_{63}\sigma_6\sigma_3 & \rho_{64}\sigma_6\sigma_4 & \rho_{65}\sigma_6\sigma_5 & \sigma_6^2 \end{pmatrix}$$

Time points correspond to rows and columns

$\rho_{ij}\sigma_i\sigma_j$	covariance for time points i and j
$\rho_{ij} = \rho_{ji}$	correlation between time point i and j
σ_i	standard deviation for time point i

Mahalanobis distance

The Mahalanobis distance D

- can be either computed directly on the data (*model free approach*)
- or can be computed on the parameters of a model fitted to the data (*model based approach*)

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

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

- Calculate a 90% confidence interval for the Mahalanobis distance between Ref and Test profiles
- Calculate the Mahalanobis distance between the reference data and the same profile which is shifted over 10% = D_c
- Accept global similarity if UCL(D) < D_c

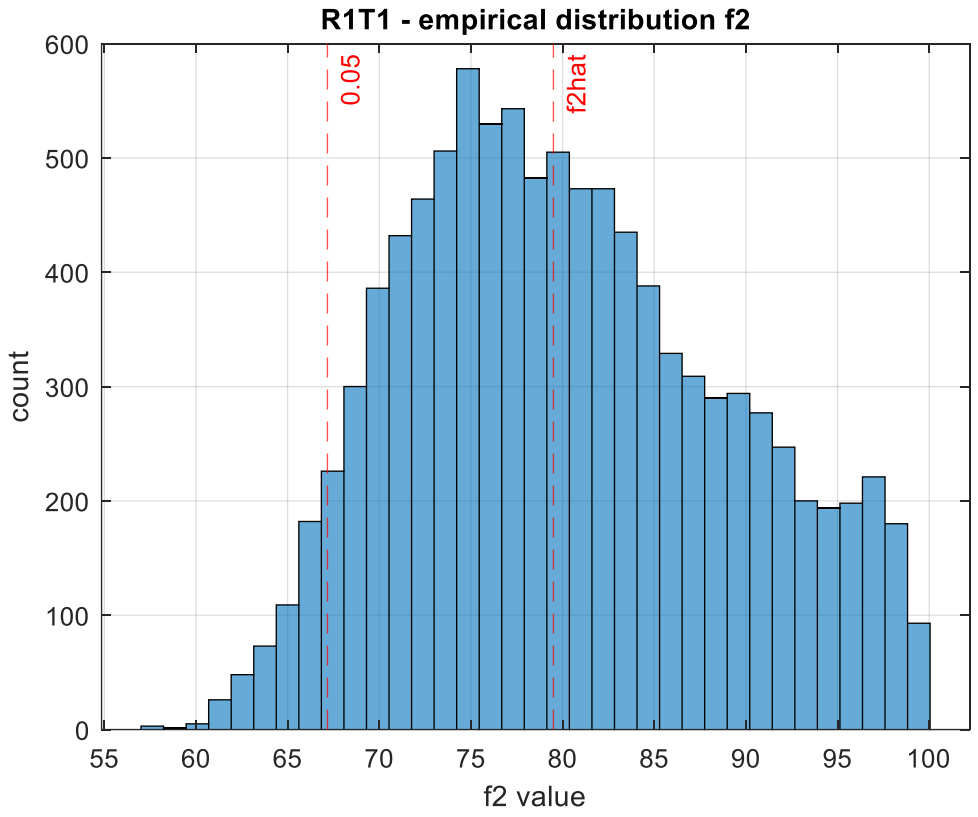
Bootstrapping – empirical distribution for f_2

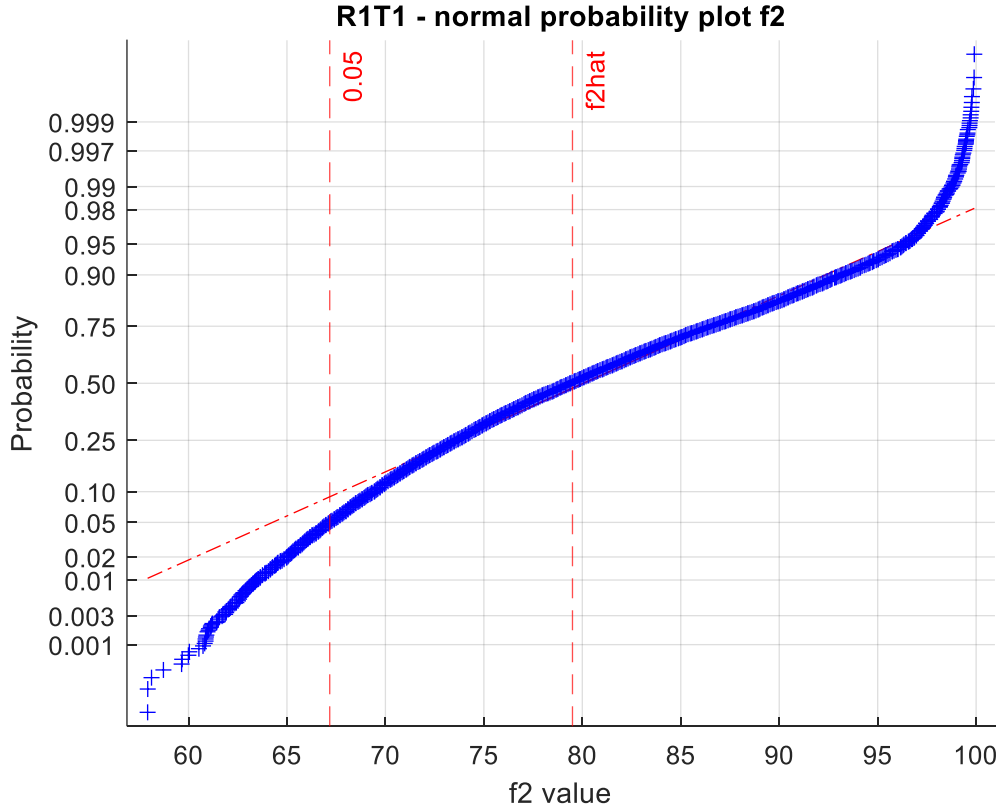
Bootstrapping is a *re-sampling* technique (*by* unit *with* replacement),
applied on observed data for both the reference batch and the test batch:

1. From the set of *12* units for the *reference* batch, *12* units are sampled
   This implies that certain units may be selected either multiple times, once, or not at all:
   starting with units (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12) subsequently 12 units are drawn
   giving as a sample (3, 9, 6, 1, 5, 10, 9, 6, 3, 6, 11, 1),
   ordered to (1, 1, 3, 3, 5, 6, 6, 6, 9, 9, 10, 11)
2. From the set of *12* units of the *test* batch, also *12* units are sampled
3. The results for the *selected* units are used to calculate an f_2 value
4. Steps 1-3 are repeated many times (10000)
5. The obtained f_2 values form an empirical probability distribution
6. The value of f_2 for which 95% of the simulated values are larger is determined,
   this is the 5th percentile which needs to be larger than **50**

Bootstrapping – empirical distribution for f_2

Calculated f_2 = 79.5, LCL = 67.2





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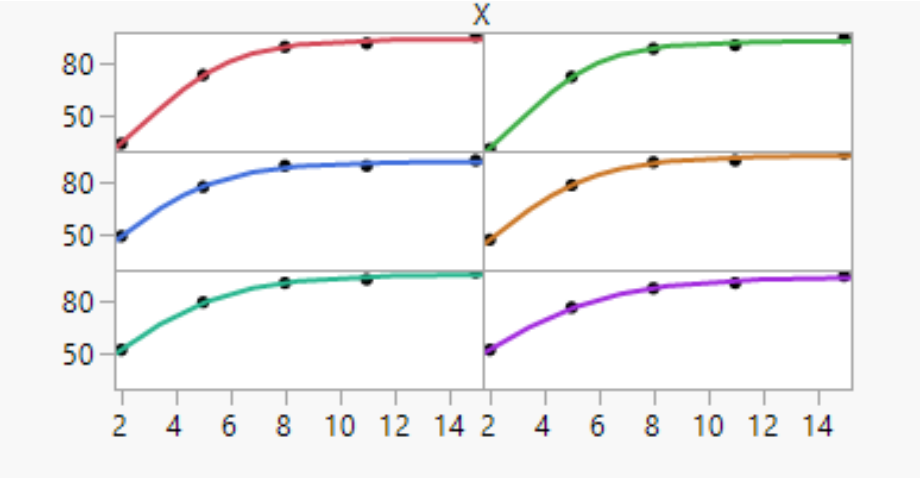
JMP 17 – what is available?

- Specialized Modelling
  - Curve Fitting
    - Dissolution Curve Analysis
      - Higuchi Curves
      - Hixson-Crowell Curves
      - Korsmeyer-Peppas Curves
      - Sigmoid Curves
      - **Model-Free Comparisons**
        - f_1 analysis
        - f_2 analysis (including bootstrapping)
        - Multivariate Distance (= Mahalanobis distance)



Regulatory guidance – **EMA** & AAPS

EMA Clinical pharmacology and pharmacokinetics:
Questions & Answers
3. Bioequivalence (general)

- EMA Q&A 3.09 (Sep 2018)
  - Do not use the Mahalanobis distance, instead use bootstrapping of f_2
- EMA Q&A 3.11 (Feb 2022)
  - Use bootstrapping of E(f_2)
- EMA Q&A 3.13 (Aug 2023)
  - Further requirements study design

Regulatory guidance – **EMA** & AAPS

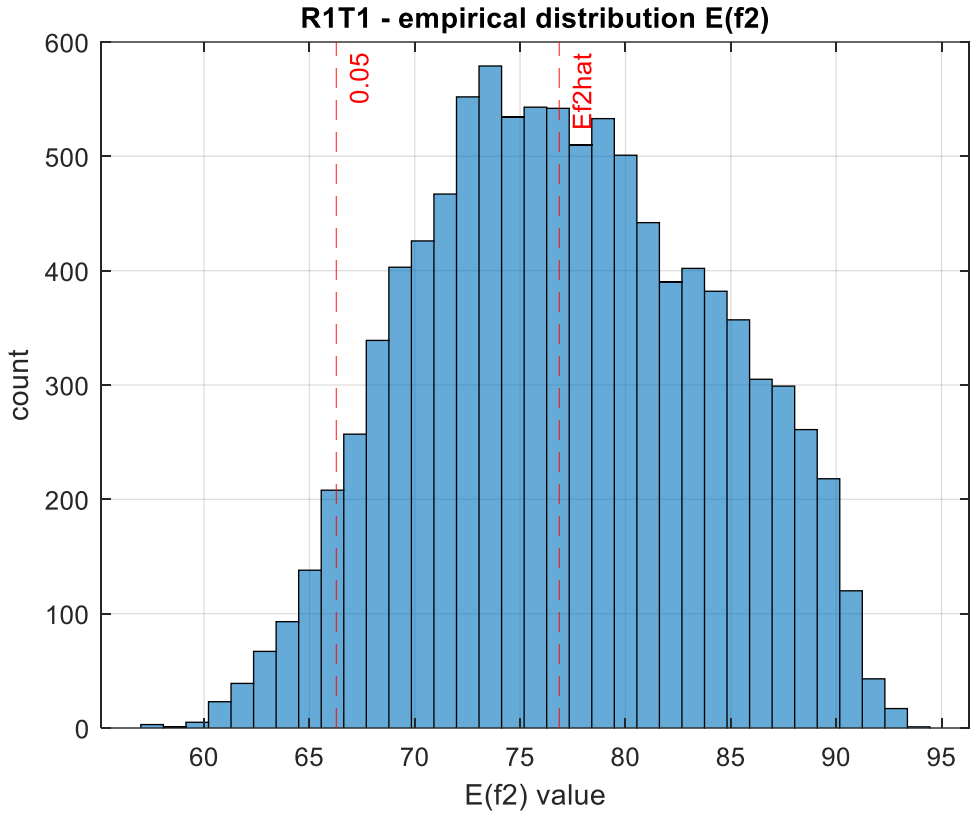
- EMA Q&A 3.11 (Feb 2022)
  - Use bootstrapping of E(f_2)

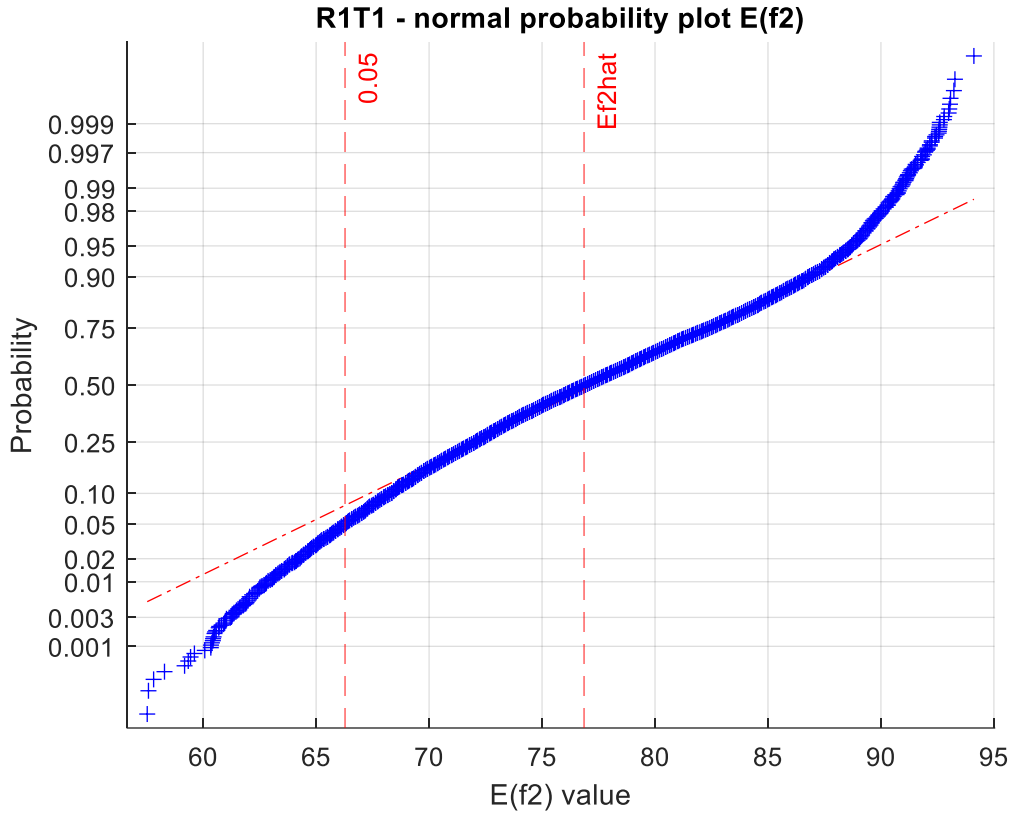
$$\hat{f}_2 = 100 - 25 \times log\left[1 + \frac{\sum_{i=1}^{P}(\bar{R}_i - \bar{T}_i)^2}{P}\right]$$

$$E(\hat{f}_2) = 100 - 25 \times log\left[1 + \frac{\sum_{i=1}^{P}(\bar{R}_i - \bar{T}_i)^2}{P} + \frac{\sum_{i=1}^{P}\left(\frac{s_{R_i}^2}{n_{R_i}} + \frac{s_{T_i}^2}{n_{T_i}}\right)}{P}\right]$$

Bootstrapping – empirical distribution for $E(f_2)$

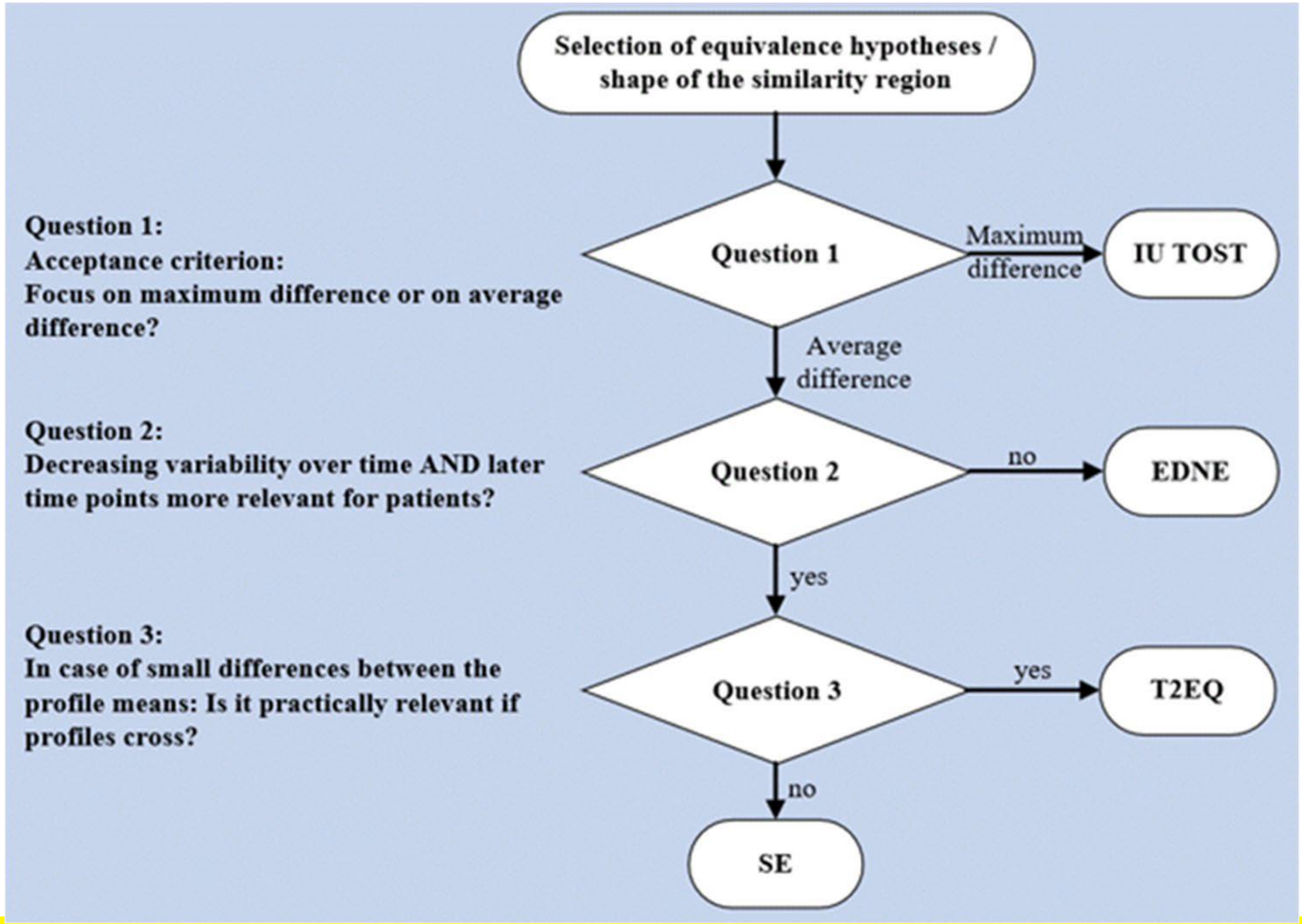
Calculated $E(f_2)$ = 76.9, LCL = 66.3 Calculated f_2 = 79.5, LCL = 67.2





Regulatory guidance – EMA & **AAPS**

The '**AAPS**' working group proposes the following decision tree



Q1: local vs. global similarity

Q2: related to pharmacokinetics (IVIVC)

Q3: related to statistical power when profiles cross

(read paper for full understanding)

Regulatory guidance – EMA & **AAPS**

Metrics proposed by the 'AAPS' working group

Dissolution testing and linear algebra

- Covariance matrices
  - Variances are on the diagonal
  - Covariances are on the lower and upper off-diagonal parts

- Mahalanobis distance (T2EQ)
- Penrose distance (SE.EQ)
- Euclidean distance (EDNE.EQ)

$$\Sigma = \begin{pmatrix} \sigma_1^2 & \rho_{12}\sigma_1\sigma_2 & \rho_{13}\sigma_1\sigma_3 & \rho_{14}\sigma_1\sigma_4 & \rho_{15}\sigma_1\sigma_5 & \rho_{16}\sigma_1\sigma_6 \\ \rho_{21}\sigma_2\sigma_1 & \sigma_2^2 & \rho_{23}\sigma_2\sigma_3 & \rho_{24}\sigma_2\sigma_4 & \rho_{25}\sigma_2\sigma_5 & \rho_{26}\sigma_2\sigma_6 \\ \rho_{31}\sigma_3\sigma_1 & \rho_{32}\sigma_3\sigma_2 & \sigma_3^2 & \rho_{34}\sigma_3\sigma_4 & \rho_{35}\sigma_3\sigma_5 & \rho_{36}\sigma_3\sigma_6 \\ \rho_{41}\sigma_4\sigma_1 & \rho_{42}\sigma_4\sigma_2 & \rho_{43}\sigma_4\sigma_3 & \sigma_4^2 & \rho_{45}\sigma_4\sigma_5 & \rho_{46}\sigma_4\sigma_6 \\ \rho_{51}\sigma_5\sigma_1 & \rho_{52}\sigma_5\sigma_2 & \rho_{53}\sigma_5\sigma_3 & \rho_{54}\sigma_5\sigma_4 & \sigma_5^2 & \rho_{56}\sigma_5\sigma_6 \\ \rho_{61}\sigma_6\sigma_1 & \rho_{62}\sigma_6\sigma_2 & \rho_{63}\sigma_6\sigma_3 & \rho_{64}\sigma_6\sigma_4 & \rho_{65}\sigma_6\sigma_5 & \sigma_6^2 \end{pmatrix}$$

$$\Sigma = \begin{pmatrix} \sigma_1^2 & 0 & 0 & 0 & 0 & 0 \\ 0 & \sigma_2^2 & 0 & 0 & 0 & 0 \\ 0 & 0 & \sigma_3^2 & 0 & 0 & 0 \\ 0 & 0 & 0 & \sigma_4^2 & 0 & 0 \\ 0 & 0 & 0 & 0 & \sigma_5^2 & 0 \\ 0 & 0 & 0 & 0 & 0 & \sigma_6^2 \end{pmatrix}$$

$$\Sigma = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix}$$

JMP 17 – what is missing?

- EMA Bootstrapping $\mathbf{E}(f_2)$
  - 85% rule *at* the bootstrap sample level
  - documentation
- AAPS metrics
  - T2EQ claimed to overcome objections against Mahalanobis distance
  - SE.EQ crossing profiles
  - EDNE.EQ valid statistical alternative to f_2, later time points more relevant

Routines are available as R packages and as in house developed Matlab code, but these lack easy access as they require programming skills and the corresponding programming platforms.

How to get new functionality in JMP?

JMP WISH LIST AND EARLY ADOPTER PROGRAM

1. Ask for new functionality by adding it to the
   JMP Wish List - JMP User Community
   https://community.jmp.com/t5/JMP-Wish-List/idb-p/jmp-wish-list

2. Look for new functionality in pre-release versions of JMP
   as participant in the 'Early Adopter' program (by invitation)
   let's users have a voice in the development process

JMP 18 wish list

0

dissolution testing in compliance with EMA + more

Status: Investigating • Submitted by Piet_Hoogkamer on 08-23-2023 10:11 AM • 4 Comments (4 New)

What inspired this wish list request?
Comparative dissolution testing is supported by JMP 17.2.0 standard edition, but the offered functionality is not in compliance with the latest EMA guidance.
Recent proposed metrics by Hoffelder (AAPS 2023 paper) are not available.

What is the improvement you would like to see?
Next to bootstrapping of the expected value of f2, in compliance with EMA rules (August 2023), metrics proposed by Hoffelder should preferably be added (T2EQ, SE and EDNA).

Why is this idea important?
Currently, the mentioned functionality is created in R and Matlab code, only accessible to a few people. Having this functionality in JMP will avoid the need for programming skills and additional programming platforms. This will bring the analysis options in reach of the experimenters who measured the data.
So, it is about ease of use and for pharma professionals a must have.

4 Comments

SamGardner STAFF

Status changed to: Acknowledged

@PI we plan to provide the T2EQ method in JMP version 18, based on the papers by Hoffelder. We will look into the other methods listed.

New functionality in JMP 18 EA6

- *New T2EQ for Dissolution Similarity tool for comparing dissolution curves in Fit Curve.*
  - *Tell us more: Is the T2EQ well-known, or is it gaining momentum?*
    Is Curve DOE useful for T2EQ?
    Would you want to define your own equivalence margin,
    or is the default 10% difference sufficient?

Comparative dissolution testing - conclusions

- Debate on what is the most appropriate approach is still going on
- JMP offers *limited* functionality, introduced in version 17.
  This functionality is *not* supporting EMA requirements *nor* AAPS recommendations
- Extension of the JMP functionality has been requested, and version 18 should bring improvements, however, the early adopter version does unfortunately *not* bring that much.

Any questions?

Literature Comparative Dissolution Testing

EMA https://www.ema.europa.eu/en/human-regulatory/research-development/scientific-guidelines/clinical-pharmacology-pharmacokinetics/clinical-pharmacology-pharmacokinetics-questions-answers#3.-bioequivalence-(general)-section

A series of articles published in 'The AAPS Journal' tell the outcome of a workshop held on May 21-22, 2019, at the University of Maryland, Baltimore, entitled:
"In Vitro Dissolution Similarity Assessment in Support of Drug Product Quality: What, How, When".

0.	Agenda and Presentations	https://www.pharmacy.umaryland.edu/centers/cersievents/dissolution-similarity/
1.	Workshop Summary Report	The AAPS Journal (2020) 22:74
2.	Requirements and Global Expectations	The AAPS Journal (2022) 24:50
3.	Statistical Principles, Methods and Considerations	The AAPS Journal (2022) 24:54
4.	Best Practices, Decision Trees and Global Harmonization	The AAPS Journal (2023) 25:44

The workshop was attended by 160 scientists from academia, pharmaceutical companies (Merck, BMS, Boehringer, Pfizer, Eli Lilly) and regulatory authorities (FDA/CDER, EMA, Health Canada, Anvisa).

Manly BFJ, Navarro Alberto JA
Multivariate Statistical Methods – A Primer – 4th Ed.
CRC Press, 2017, Boca Raton (Fl)

Manly BFJ
Randomization, Bootstrap and Monte Carlo Methods in Biology – 3rd Ed.
Texts in Statistical Science
CRC Press, 2007, Boca Raton (Fl)

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