

Long-term stability investigation of macromolecules in an isochronic study design

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EBF IGM long-term stability team***

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Introduction

*“Drug stability in a biological fluid is a function of the storage conditions, the chemical properties of the drug, the matrix, and the container system” **

*“Stability should be ensured for every step in the analytical method, meaning that the conditions applied to the stability tests, such as sample matrix, anticoagulant, container materials, storage and analytical conditions should be similar to those used for the actual study samples” ***

**FDA, CDER: Guidance for industry, bioanalytical method validation (2001).*

***EMA: Guideline on bioanalytical method validation (2011).*

Content

- Guidance for long-term stability of macromolecules
- Introduction to the isochronic design
- Case studies of macromolecules in an isochronic study design
- Opportunities for the used of the isochronic study design
- EBF IGM topic team expected outcome in the future

Guidance for long-term stability*

- Stability should be ensured for every step in the analytical method, meaning that the conditions applied to the stability tests, such as sample matrix, anticoagulant, container materials, storage and analytical conditions should be similar to those used for the actual study samples.

* *EMA: Guideline on bioanalytical method validation (2011).*

Guidance for long-term stability*

- Stability of the analyte in the studied matrix is evaluated using low and high QC samples (blank matrix spiked with analyte at a concentration of a maximum of 3 times the LLOQ and close to the ULOQ) which are analysed immediately after preparation and after the applied storage conditions that are to be evaluated.
- The QC samples are analysed against a calibration curve, obtained from freshly spiked calibration standards, and the obtained concentrations are compared to the nominal concentrations.
- The mean concentration at each level should be within $\pm 20\%$ of the nominal concentration.

* EMA: *Guideline on bioanalytical method validation (2011)*.

Guidance for long-term stability*

- The QC samples should be stored in the freezer under the same storage conditions and at least for the same duration as the study samples.
- For large molecules (such as peptides and proteins) stability should be studied at each temperature at which study samples will be stored.

- *EMA: Guideline on bioanalytical method validation (2011).*

Isochronic design - background

Background

- Isochronic design based on Dadgar D., et al.(1995)*
- The experimental design is based on the assumption that the degradation of even instable compounds in biological matrices is negligible at temperatures lower than -130°C .
- Due to the storages below -130°C , the design has only been applied to small molecules so far.

* Dadgar D, Burnett Pe, Choc Mg, Gallicano K, Hooper Jw: Application issues in bioanalytical method validation, sample analysis and data reporting. *J Pharm Biomed Anal* 13(2), 89-97 (1995).
Dadgar D, Burnett Pe: Issues in evaluation of bioanalytical method selectivity and drug stability. *J Pharm Biomed Anal* 14(1-2), 23-31 (1995).

Isochronic design – study set-up

- Prepare pools of stability QC's in 3 concentrations:
 - one close to LLOQ,
 - one close to ULOQ, and
 - one level (5-20 times) above ULOQ
- Divide into nine sub pools for each stability point for each concentration level.
- Store baseline (t_0) samples at -150°C and stability test samples (t_i , t_{ij} , etc.) at test temperature (e.g. -80°C).

Isochronic design – study set-up

- Transfer the stability test samples (t_i , t_{ij} etc.) to -150 °C after the (pre) defined time periods.
- Analysis all samples from the same concentration level in the same run at the end of the study (9 single determinations for each concentration at each time point).

	Start	Period 1	Period 2	Period 3	Analysis
-20 or -80 degrees		T1	T2	T3	➡
-150 degrees	T0	↓	↓	↓	

Isochronic design - evaluation

- Test of pools
 - Mean concentration $\pm 20\%$ of nominal concentration
- Accept of the isochronic design
 - Mean concentration of baseline (t_0) $\pm 20\%$ of nominal concentration
- Stability can be concluded, if:
 - Mean concentration of each stability timepoint $\pm 20\%$ of mean baseline concentration
 - $CI_{90\%}$ is included in the interval $\pm 25\%$

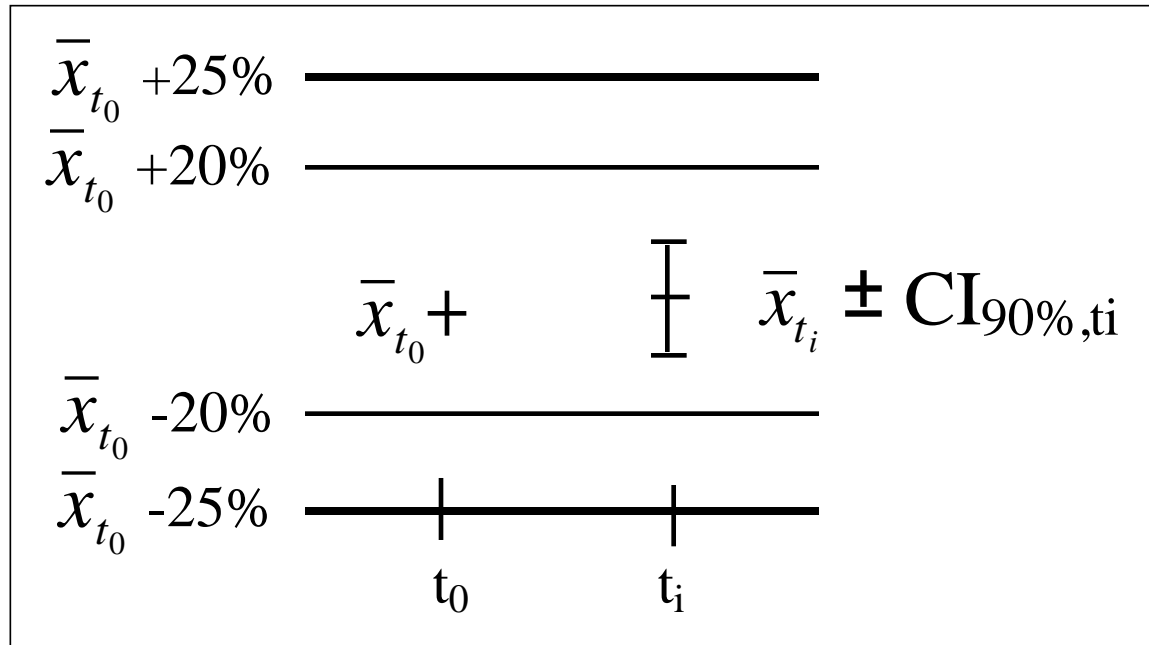
Isochronic design – statistical evaluation

Test of accuracy: $80\% \leq \left(\frac{\bar{x}_{t_i}}{\bar{x}_{t_0}} \times 100\% \right) \leq 120\%$

Test of precision ($CI_{90\%,t_i}$): $\bar{x}_{t_i} \pm \left(\frac{SD}{\sqrt{n}} \right) \times t_{(n-1)(1-0.90)/2}$

where t_i is the stability at the time i and $t_{(n-1)(1-0.90)/2}$ is the 90% t -fractile with $n-1$ degrees of freedom.

Isochronic design – statistical evaluation



Isochronic design – small molecules

- The study design has been evaluated in 144 long-term stability studies with small molecules and in seven different plasma matrices. The isochronic design has been used at Lundbeck for the past 8 years for regulated bioanalysis.
- The isochronic approach has provided conclusive stability data for all conducted studies.

- *Susanne Pihl, Anna Karina Trap Huusom, Morten Rohde, Mette Nøhr Poulsen, Martin Jørgensen and Morten A. Kall. Evaluation of an isochronic study design for long-term frozen stability investigation of drugs in biological matrices, Bioanalysis 2(6), 1041-49, (2010)*

Isochronic design – large molecules

- It was at the Crystal City III conference argued that very low temperature can cause denaturation or precipitation of matrix proteins and, thus, affect protein binding. This issue may be relevant to consider, when investigating the stability of macromolecules.*
- The applicability for macromolecules is being challenged by the group and case studies will be presented.

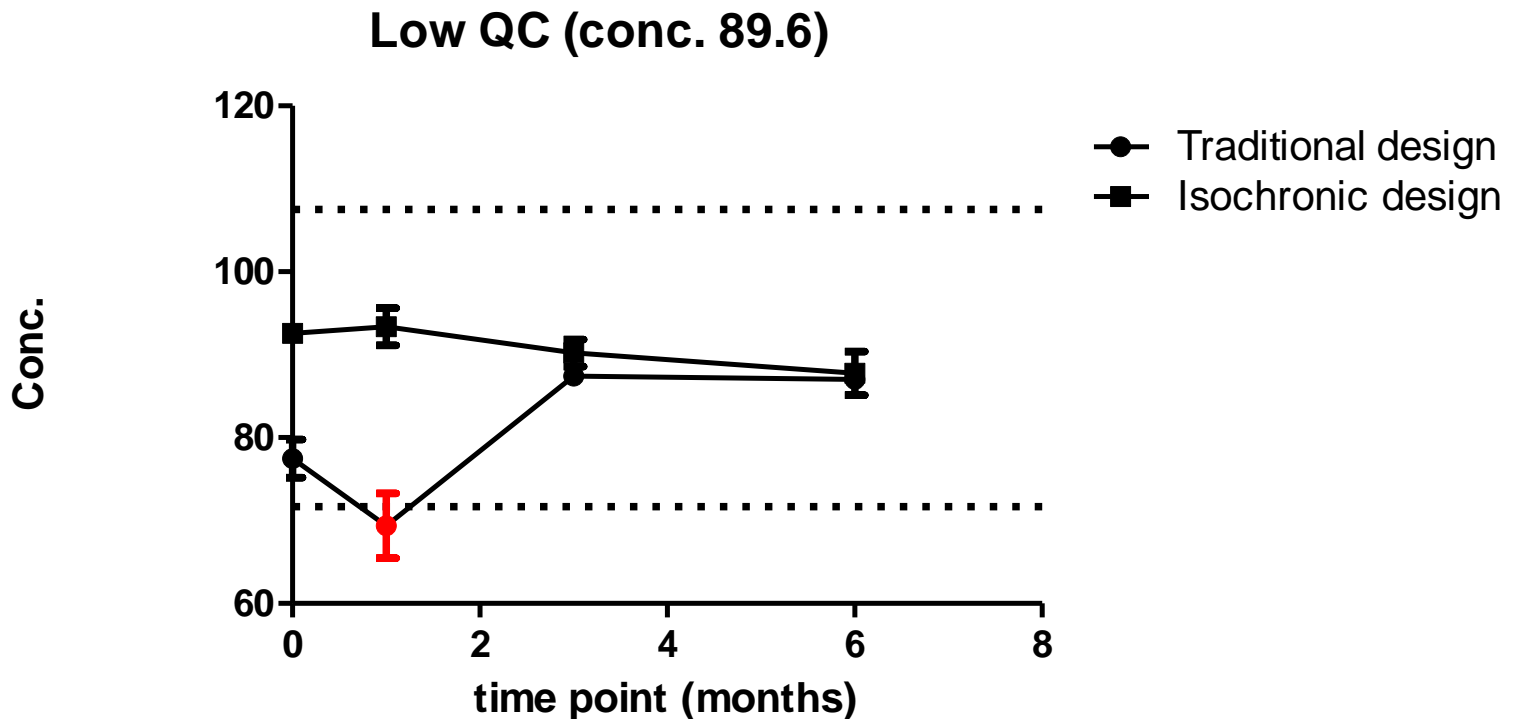
* Viswanathan C, Bansal S, Booth B *et al.*: Quantitative bioanalytical methods validation and implementation: Best practices for chromatographic and ligand binding assays. *Pharm Res* 24(10), 1962-1973 (2007)

Case study 1– Long-term stability at -20°C of a derivatised protein in mouse EDTA plasma evaluated by isochronic design and traditional design

- Isochronic design was accepted if:
 - Mean concentration of stability sample stored at -140°C for 6 months (t_0) was within $\pm 20\%$ of freshly prepared stability sample ($t_{0 \text{ fresh}}$)
 - Mean concentration of baseline (t_0) was $\pm 20\%$ of mean nominal concentration

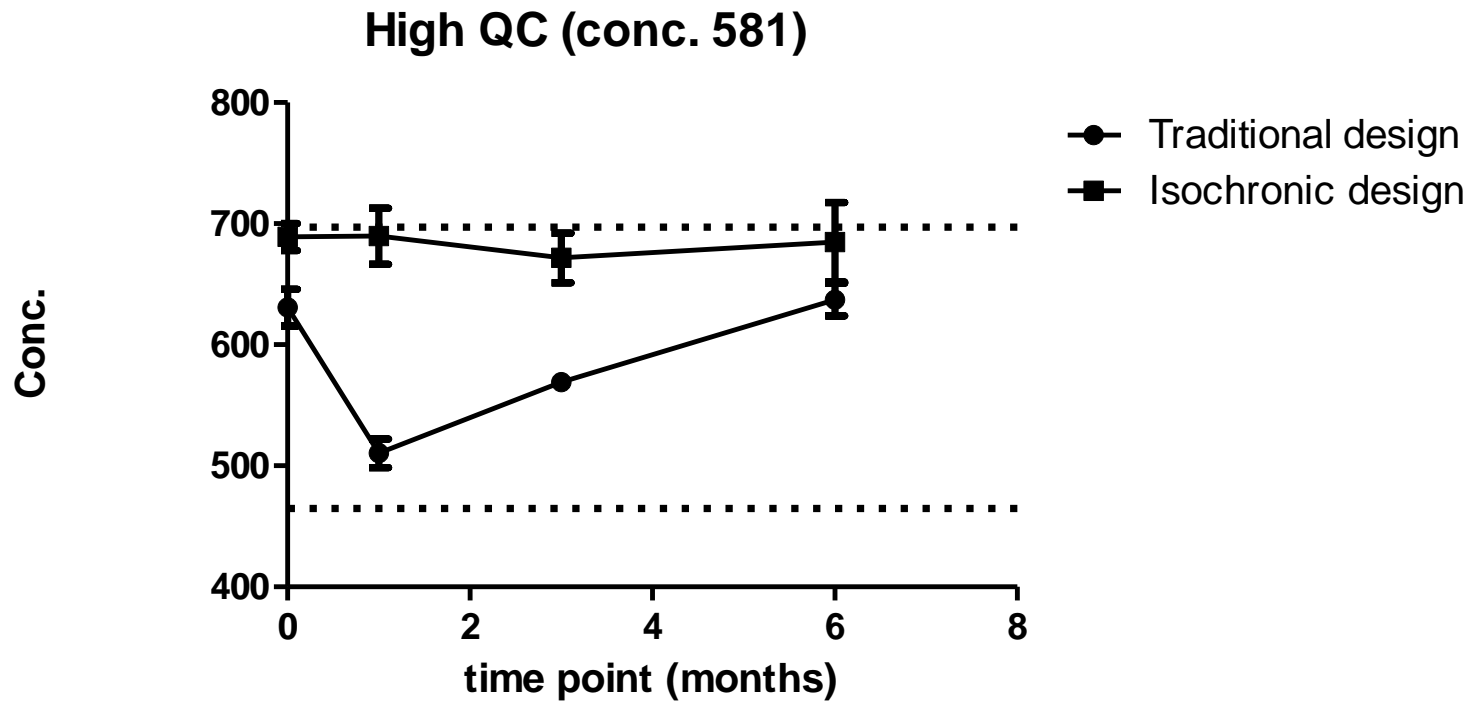
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Case study 1– Long-term stability at -20°C of a derivatised protein in mouse EDTA plasma evaluated by isochronic design and traditional design



n=3 duplicates

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Case study 2 – Long-term stability of a protein in rat EDTA plasma stored at -20°C

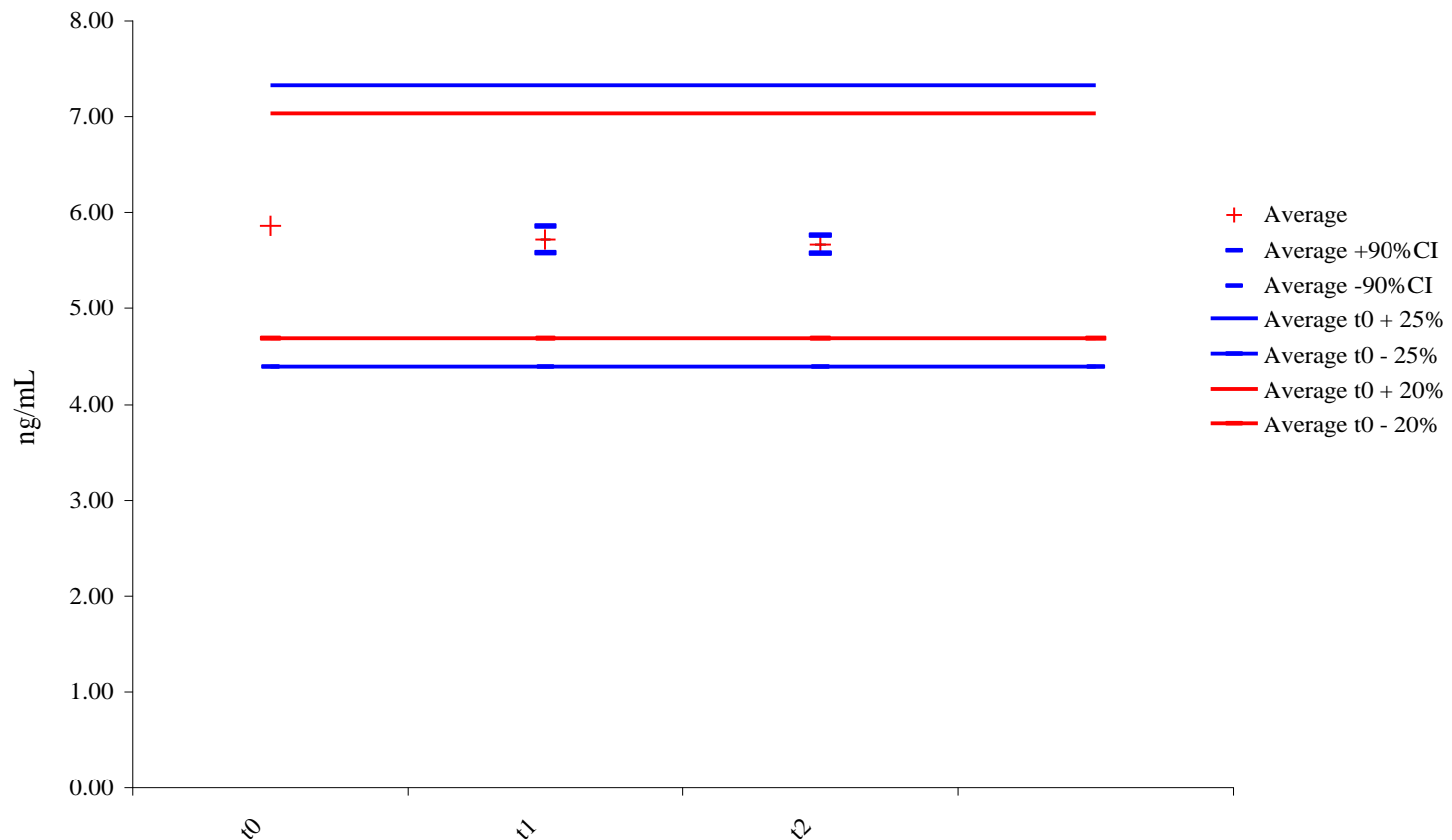
- Test of pools:
 - All concentration levels within $\pm 20\%$ of nominal concentration
- Test of design:
 - Baseline samples were 105 – 117% of nominal concentration
- Test of long-term stability

	LOW (0.45 ng/mL)		MEDIUM (5 ng/mL)		HIGH (10000 ng/mL)	
	t ₁	t ₂	t ₁	t ₂	t ₁	t ₂
Accuracy (%)*	99.4	98.3	97.6	96.8	118	116
Precision (%)**	98.3-101	96.8-99.7	95.3-99.9	95.2-98.4	115-121	113-119

* Mean concentration of each stability timepoint $\pm 20\%$ of mean baseline concentration

** CI90%, is included in the interval $\pm 25\%$

Case study 2 – Long-term stability of a protein in rat EDTA plasma stored at -20°C



Nominal concentration: 5 ng/mL, t_1 : 3 weeks and t_2 : 5 weeks

Isochronic design - Opportunities

- Long-term stability testing of anti-drug antibodies in the biological matrix.
- Testing of Stability Incurred Stability (ISS).
- Testing of reagents

EBF IGM expected outcome

- A formal topic team (TT21) has been established.
- Test further macromolecules to be able to come up with a recommendation from EBF
- Goal to issue a EBF position paper regarding long-term stability of macromolecules

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 - Daniela Stoellner, Novartis