Accelerated Stability of Peptides

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Considerations for Stability of Small Molecule vs. Proteins

Small Molecules

- Subject to chemical modifications such as oxidation, deamination, hydrolysis
- Chemical modifications generally lead to loss of potency
- Only concerned with primary structure
- Often shelf-life limited by formation of low levels of degradants
- Stability indicating analytical method feasible

Proteins

- Subject to chemical modifications such as oxidation, deamination, hydrolysis
- Chemical modifications may or may not impact activity
- Impact on 1°, 2°, 3° and 4° structure must be considered
- Small changes in structure can have a large impact on activity and aggregation state
- Requirement for a panel of assays for determination of a stability indicating profile

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Considerations for Stability of Small Molecule vs. Proteins

Small Molecules

- Follow Arrhenius behavior in solution and modified Arrhenius behavior in solid state

Proteins

- Undergo multiple reversible and irreversible steps making Arrhenius behavior more difficult to detect even in solution

Can the humidity modified Arrhenius equation be utilized to effectively model protein stability?

- Initial approach - evaluate peptides
  - Chemical changes more likely to impact stability
  - Less likely to have complex higher order structure
  - Can track using a single analytical method (HPLC)

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Can ASAP be Used to Model Shelf-life of Biologics?

Goal: Determine the applicability of the **A**ccelerated **S**tability **A**ssessment **P**rogram (**ASAP**) to rapidly model the shelf-life of a peptide

- Samples are incubated at elevated temperatures and RHs
- Times to specification limits (isoconversion times) are determined
- Data are fit to the humidity modified Arrhenius equation

collision frequency

humidity sensitivity factor

$$\ln k = \ln A - E_a/(RT) + B(RH)$$

isoconversion time

activation energy

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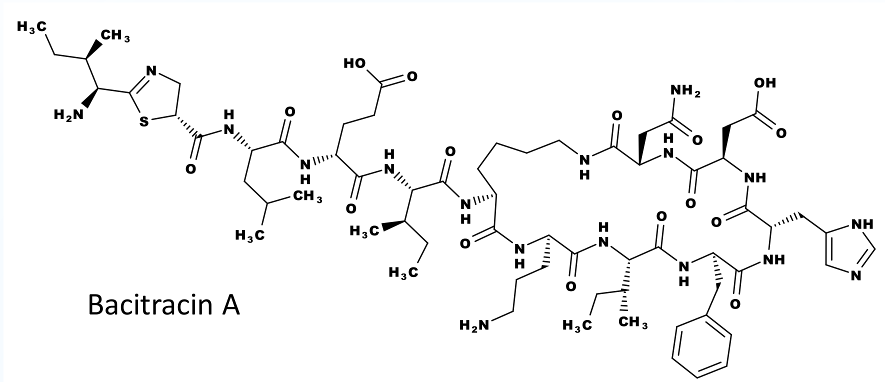
Bacitracin



- Bacitracin is a mixture of related cyclic peptides produced by *Bacillus licheniformis* and *Bacillus subtilis*
  - Inhibits the incorporation of peptidoglycan building blocks into the cell walls of gram positive bacteria
  - In broad use as a topical anti-bacterial agent
- Bacitracin A is a major component of bacitracin
  - Binding of a divalent cation such as zinc required for potent antibiotic activity
  - In metal-free form, inhibits bacterial subtilisin-type proteases

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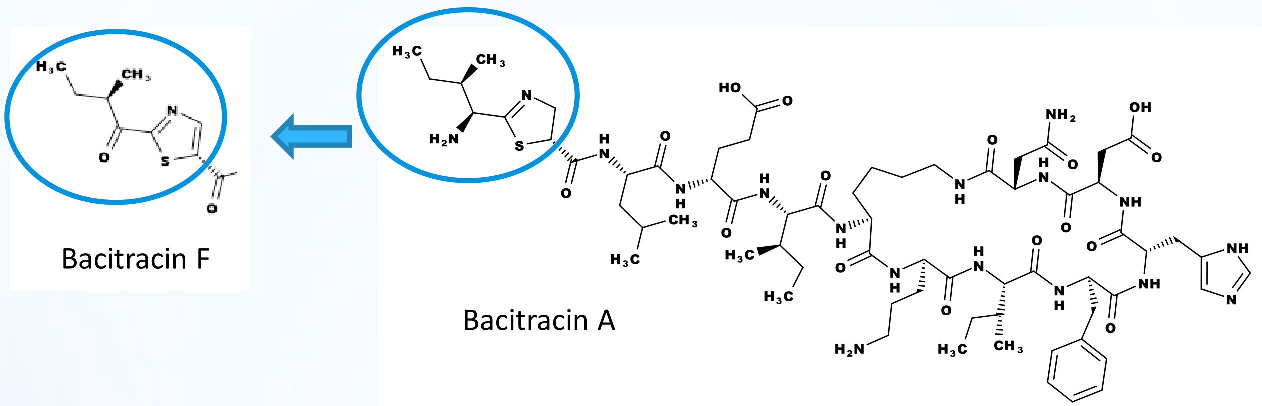
Bacitracin



Bacitracin F

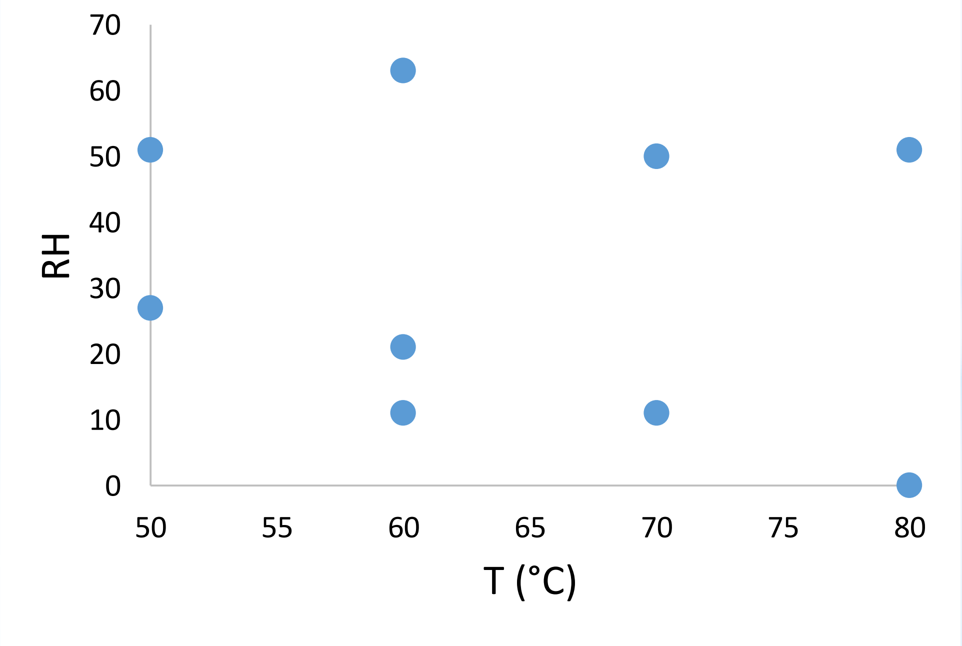
Bacitracin A

- Bacitracin F is a significant degradation product
    - Formed through oxidative deamination of the amino-thiazoline ring
    - Lacks antibiotic activity

Design of ASAP Study

- ASAP study performed comparing stability of solid bacitracin and bacitracin zinc
  - Evaluated stability indicating parameters by HPLC including:
    - Loss of bacitracin A (potency) and
    - Growth of bacitracin F (purity)

ASAP temperature and relative humidity conditions



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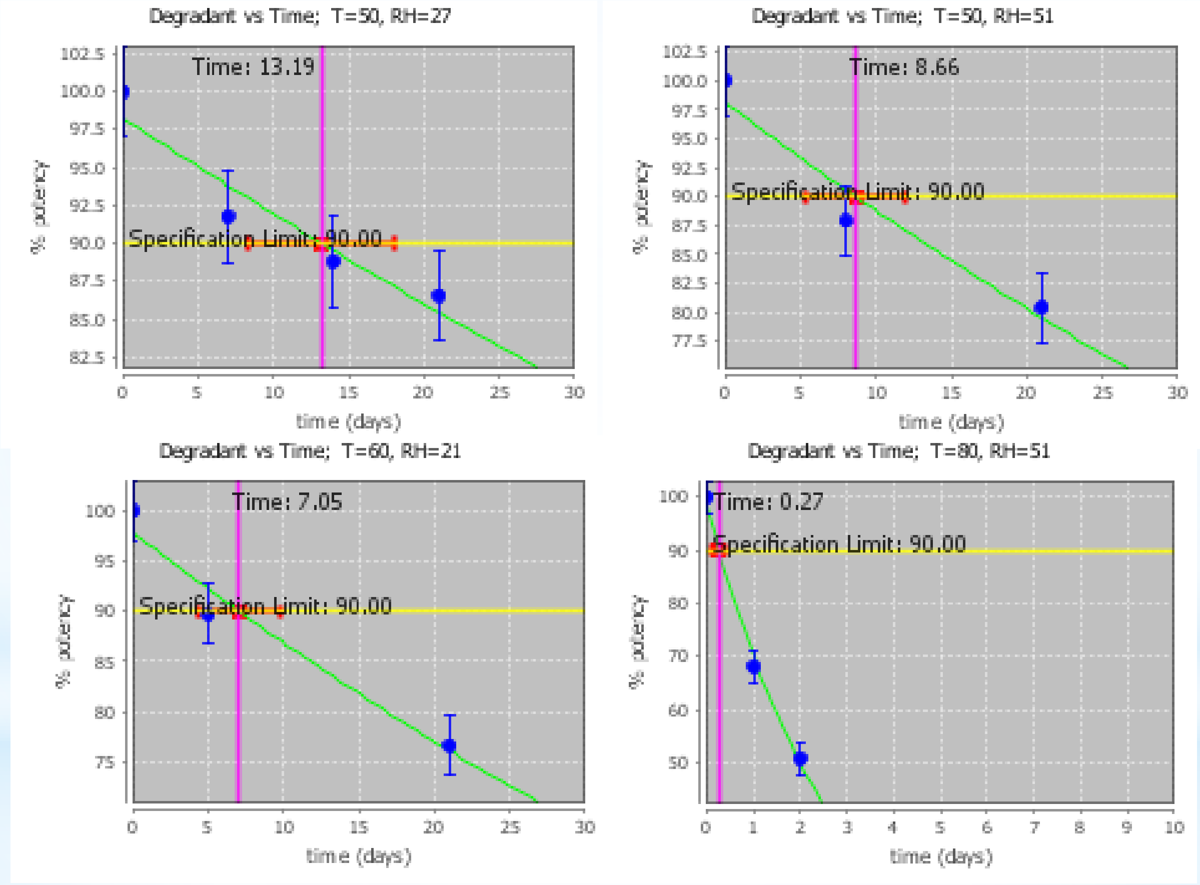
Bacitracin A Loss:
Determination of Isoconversion Times



Loss of bacitracin A followed first order kinetics

Bacitracin A Loss: ASAP Model Parameters

Good fit to modified Arrhenius equation

More sensitive measure of fit than R^2

Peptide form	ln A	E_a (kcal/mol)	B	R^2	Q^2	Mean predicted shelf-life 25°C/60% RH (open)
Bc	38.6±5.9	25.7±4.0	0.031±0.009	0.98	0.96	0.5 years
BcZn	34.3±7.6	24.2±5.2	0.010±0.010	0.94	0.87	10.4 years

Decreased collision frequency for BcZn: Zn complex- less mobility?

BcZn has lower sensitivity to moisture

BcZn is significantly more stable than Bc

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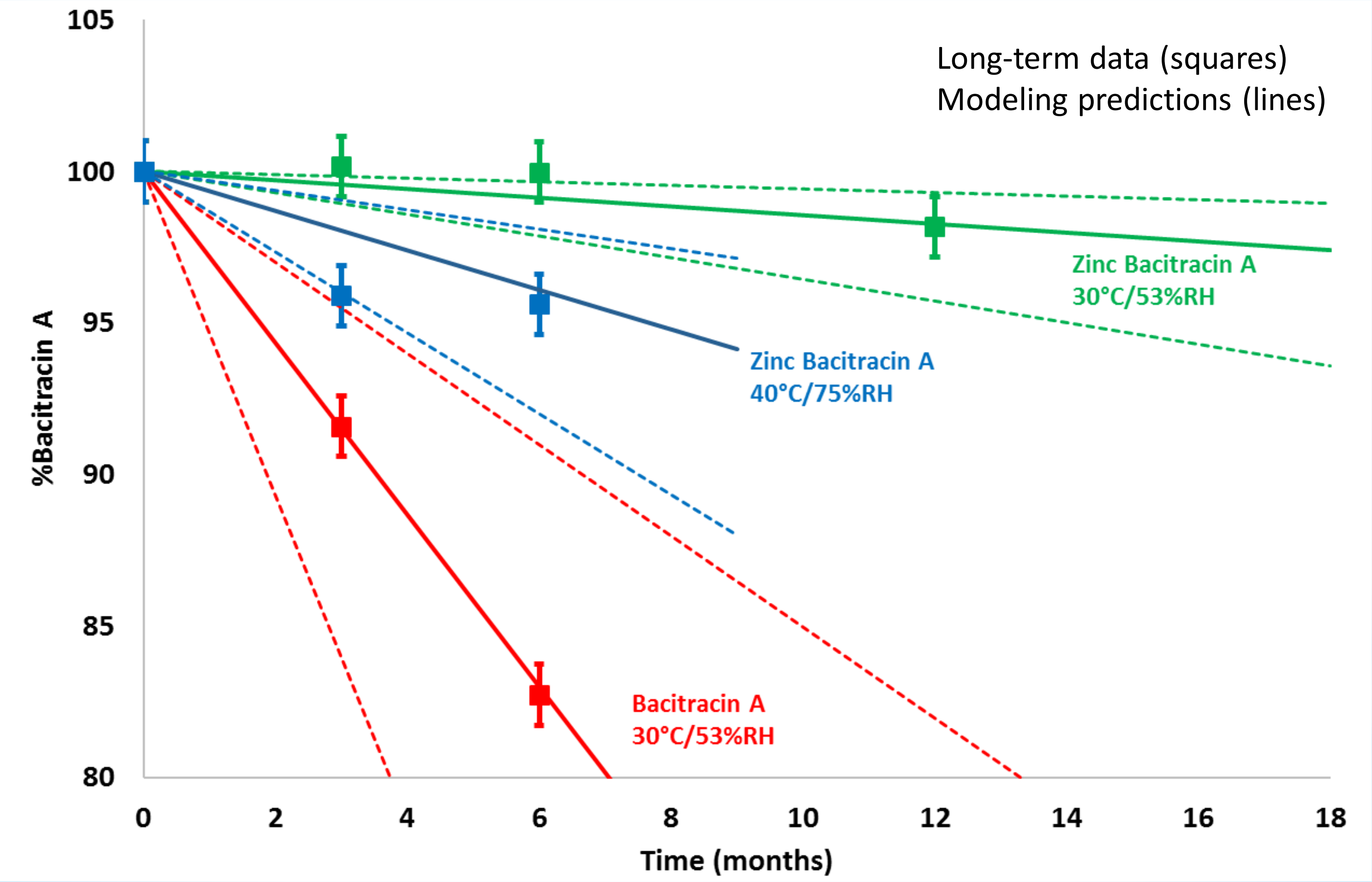
Bacitracin A Loss: Good Fit to Long Term Data



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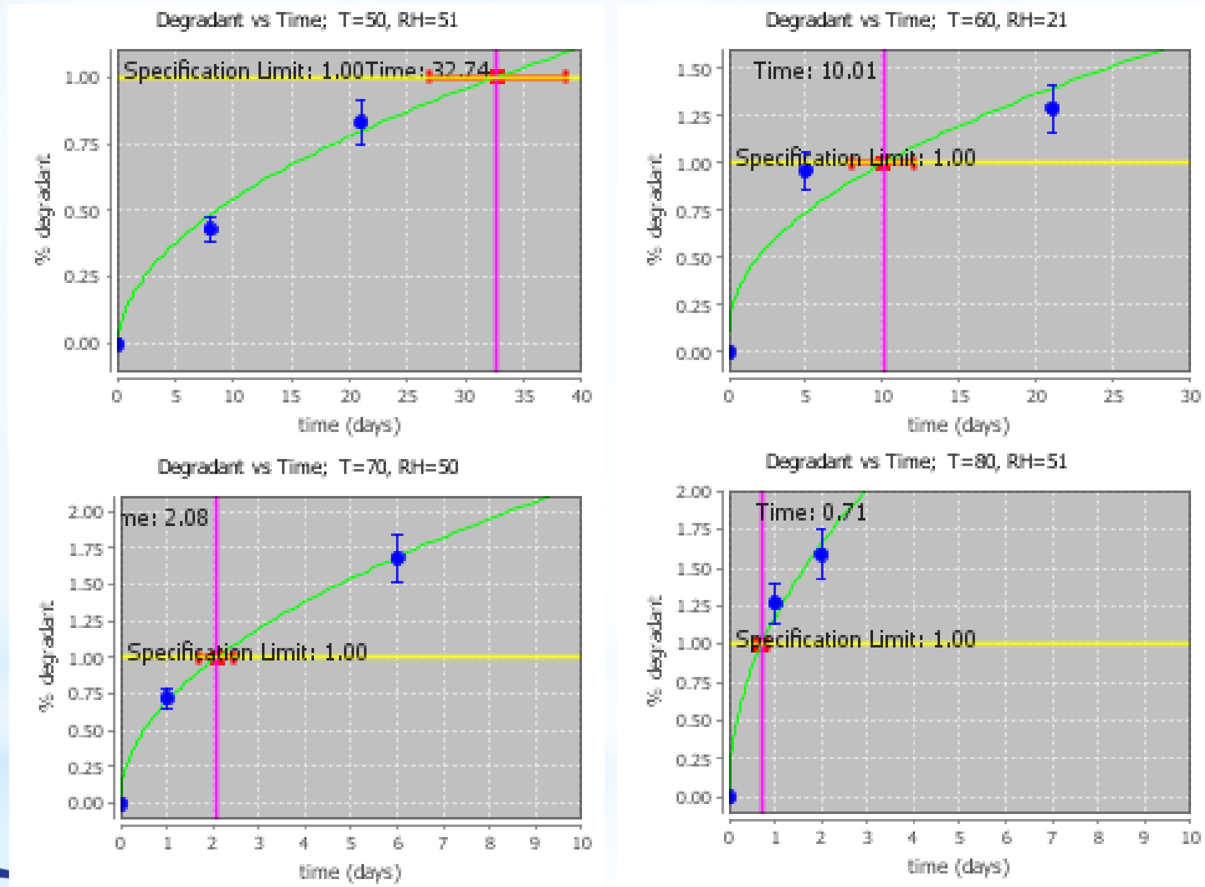
Growth of Bacitracin F: Determination of Isoconversion Times



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Growth of bacitracin F from bacitracin Zn followed diffusion kinetics

Growth of Bacitracin F: ASAP Model Parameters

Good fit to modified Arrhenius equation

More sensitive measure of fit than R^2

Peptide form	ln A	E_a (kcal/mol)	B	R^2	Q^2	Mean predicted shelf-life 25°C/60% RH (open)
Bc	44.6±4.9	29.2±3.3	0.021±0.007	0.95	0.83	1.0 month
BcZn	45.8±2.2	32.0±1.5	0.008±0.003	0.98	0.96	6.6 years

Increased activation energy for BcZn

BcZn has lower sensitivity to moisture

BcZn is significantly more stable than Bc

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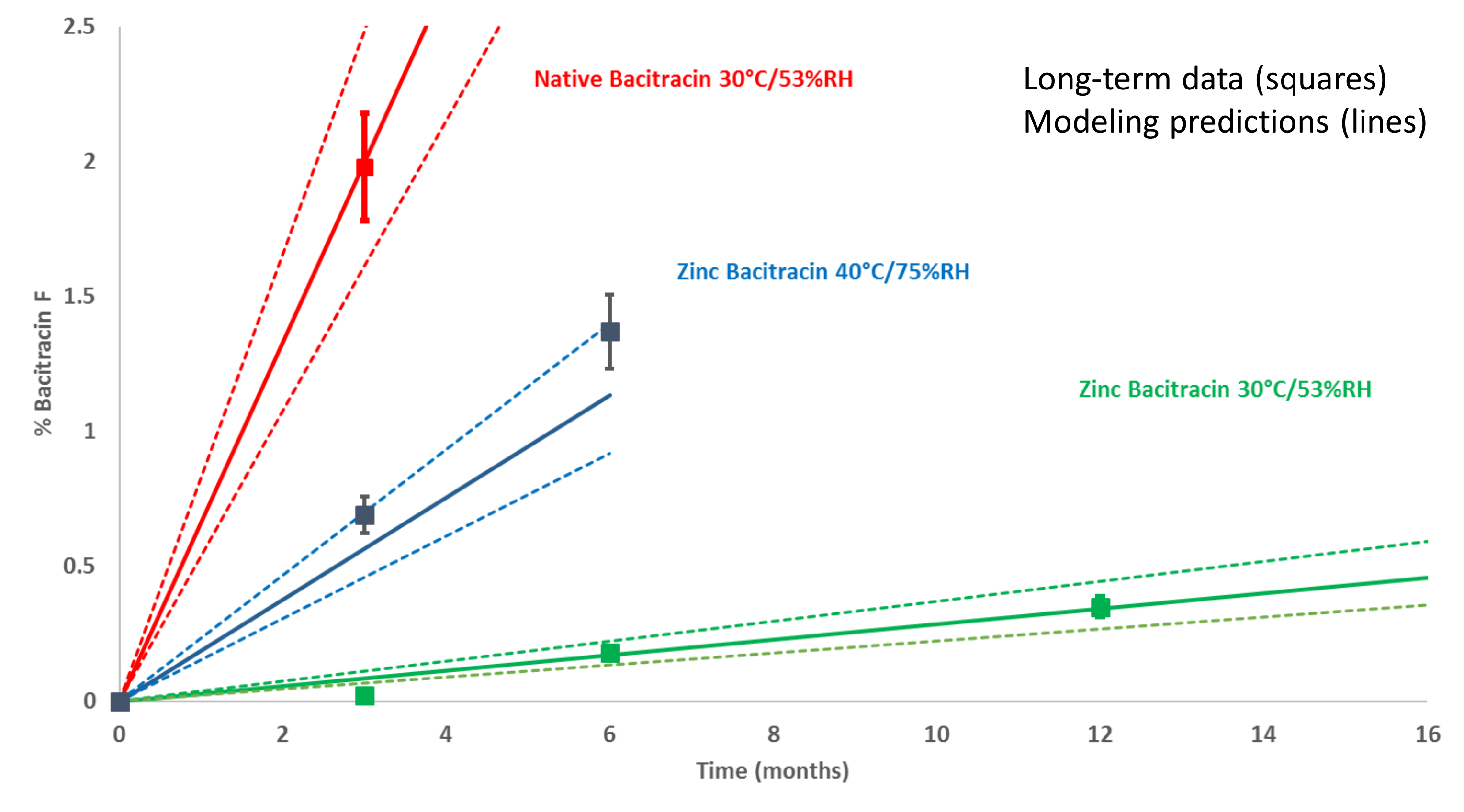
Growth of Bacitracin F: Good Fit to Long Term Data



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Summary

- The Accelerated Stability Assessment Program (ASAP) was successfully applied to a peptide for the first time
- Bacitracin and its zinc complex were exposed to a range of temperatures and humidities for up to 21 days and both loss of bacitracin A and formation of bacitracin F were analyzed by HPLC
- Model fitting to the humidity-corrected Arrhenius equation was good
- Bacitracin zinc was predicted to be significantly more stable than bacitracin
- Model predictions matched long term data validating ASAP for the determination of long term stability of a peptide
- ASAP approach could be used to greatly accelerate the drug development of peptides and potentially other biologics

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