
Development and Validation of HPLC-Corona-CAD/UV Stability Indicating Methods

Dr. Michael Swartz, Ph. D.
And
Mark Emanuele
Synomics Pharma Services

PittCon'09

mswartz@synomicspharma.com

Presentation Outline

- Forced Degradation Study Experimental Design Considerations
 - General and Specific Conditions
- Stability Indicating Method
 - Criteria and Development
- Case Study
 - Corona CAD



Forced Degradation (Chemical Stress) Studies

- Why Do a Forced Degradation Study?
 - Understand the reactive chemistry of the drug substance
 - Help anticipate future stability issues of both drug substance and drug product
 - Provides useful information for formulation and stability
 - May be required for regulatory submissions
- Forced degradation or stress testing is undertaken to demonstrate specificity when developing stability indicating methods
 - Generates a sample for method development, stability support
 - Performed prior to implementation of stability studies

Forced Degradation Experimental Design Considerations

- Drug Substance Vs. Drug Product
 - Chemistry Vs. Packaging

- Single Point in Time Vs. Continuous Monitoring

- Chromatographic Compatibility
 - Solvent
 - Concentration

Example Conditions for Forced Degradation

| <u>STUDY</u> | <u>CONDITIONS</u> |
|---------------------------|---|
| Acidic pH | 0.1N HCl |
| Neutral pH | pH 7.0 Phosphate Buffer |
| Basic pH | 0.1N NaOH |
| Oxidation | O ₂ Atmosphere, or H ₂ O ₂ |
| Photolysis (UV) | 1000 Watt h/M2 |
| Photolysis (Fluorescence) | 6x10 ⁶ lux h |

Goal: Degrade API 5-10%; Stability Indicating Method (SIM)

What is a Stability Indicating Method?

- A Stability Indicating Method (SIM) Is:
 - A **validated** method that can accurately and precisely quantitate the decrease of the API content due to degradation
 - Is specific for the drug substance
 - Shows a decrease in assay value (correlated to drug substance loss) due to degradation
 - Has no interference from excipients, impurities or degradation products
 - Detects and quantitates impurities and degradation products

Why Are Stability Indicating Methods Needed?

- It's good science
- Predominantly to support long term stability testing
 - How the quality of the drug substance or product changes over time in response to environmental factors
 - Temperature
 - Humidity
 - Light
 - Establishes storage and packaging conditions
- It's the law
 - CFR Title 21, Section 211

When to Use Stability Indicating Methods

- When Are SIMs Needed?
 - Stability Studies
 - API release
 - Drug product release
 - Toxicology dosing solutions
 - Excipient compatibility and pre-formulation
 - Packaging studies
 - Line extensions

- When Are SIMs **Not** Needed?
 - In process controls
 - Secondary assay for API
 - Titration
 - Inorganics

Implementing a Stability Indicating Method



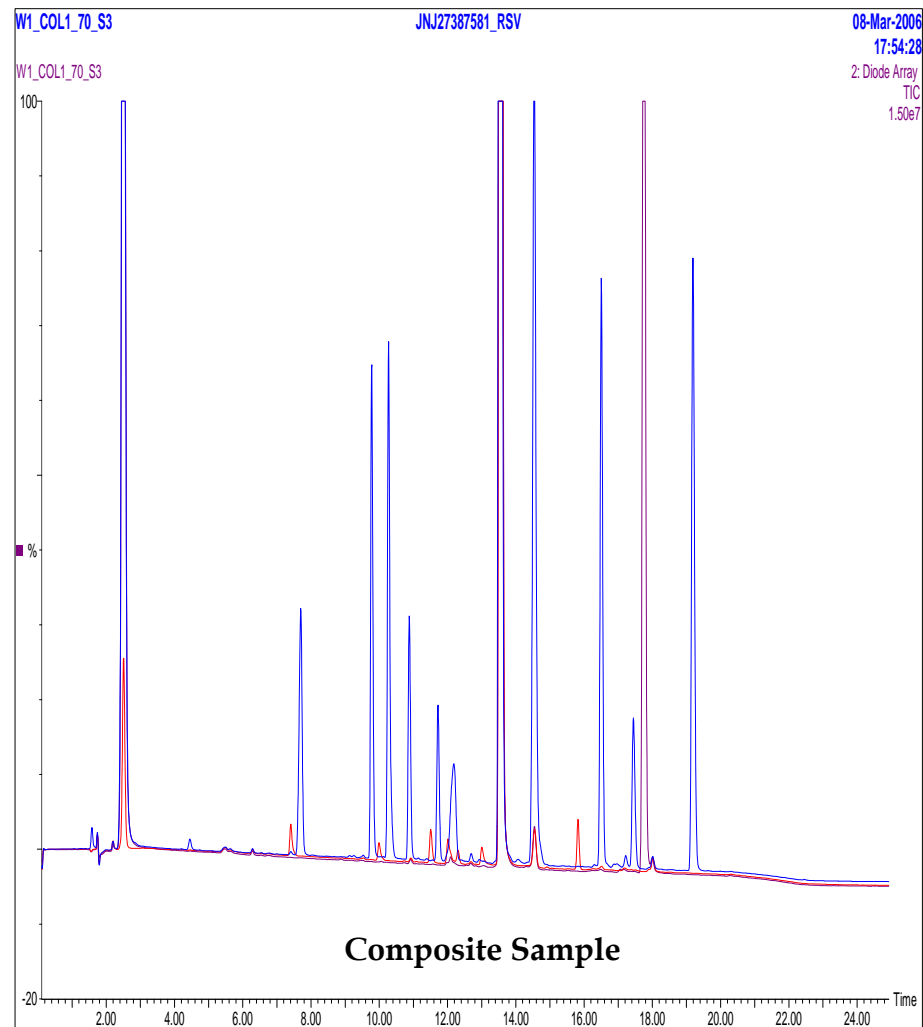
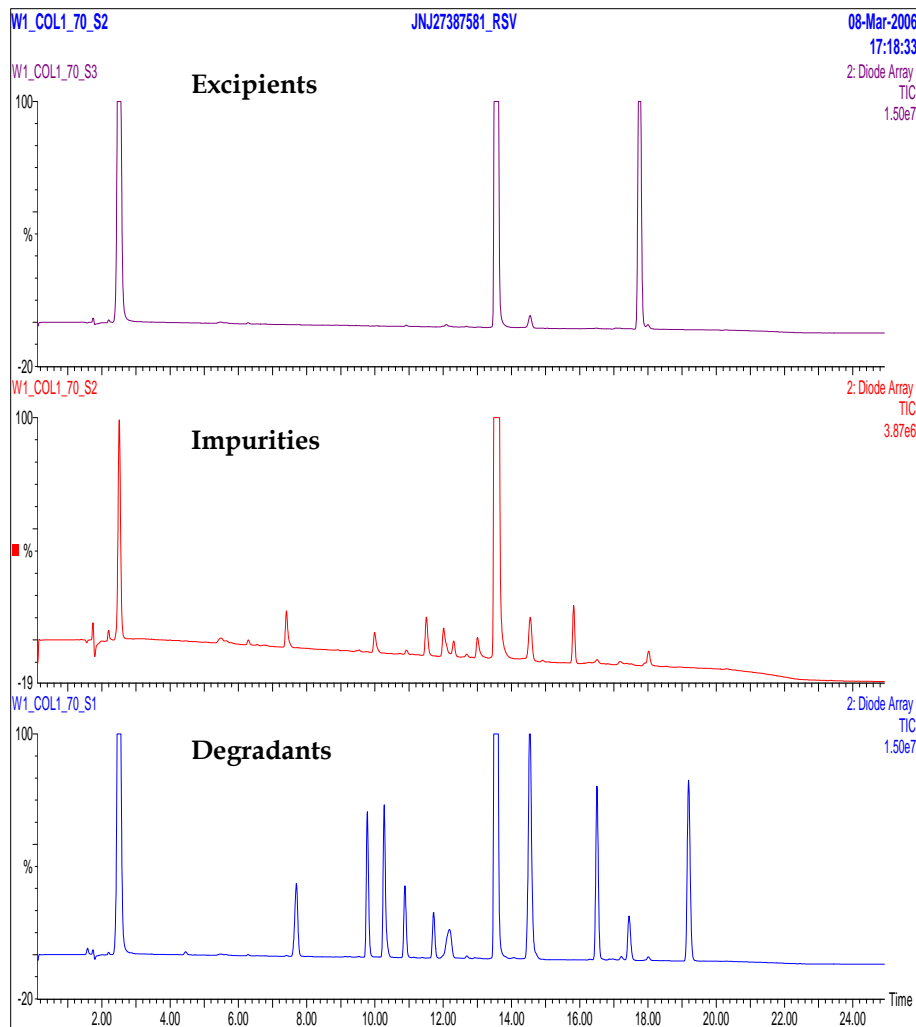
Stability Indicating Methods

Method Development Issues and Challenges

- Same as Method Development in General
 - Additional requirements
 - Resolution
 - UV Sensitivity
 - LOQ
 - Chromatographic speed
 - “Capture” intermediates
 - Column temperature control is critical

SIM Method Development Challenge

Courtesy of
Rudy Sneyers
J&J Belgium



Stability Indicating Method Development

- Manipulate Chromatographic Selectivity
 - Column
 - Mobile phase composition/type
 - pH
 - Temperature
- Specificity
 - PDA or MS
 - CAD

Goal: Baseline Resolution, No Co-elutions

Benefits and Advantages of Corona CAD

- Highest Sensitivity of “Universal” Type Detectors
- Wide Dynamic Range
- Detects any Non-volatile or Semi-volatile Compound
 - No chromophore required
- Consistent Response
- Easy to Use



Where is Corona CAD Used?

- Preformulation
 - Counterion analysis
- Process Monitoring
- Content Uniformity
- Stability
 - Degradants
- Raw Material
 - Impurities
- Cleaning Validation
 - API's and cleaning agents

Corona CAD and SIM Validation

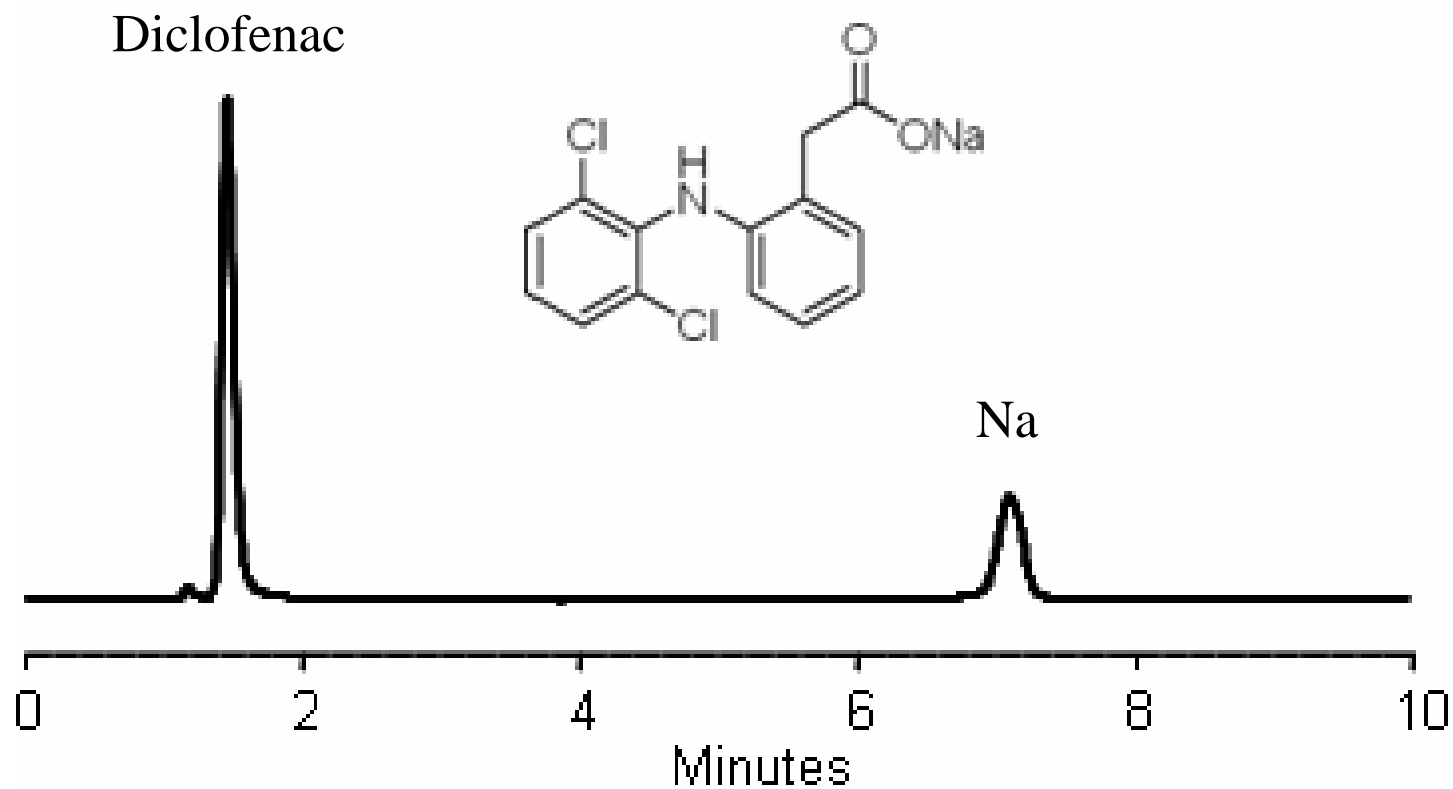
- Just Like Any Other Detector!!
 - System Suitability
 - Band Spread
 - Specificity
 - Robustness
 - Nitrogen flow
 - Gradient (if applicable)
 - Linearity
 - Precision
 - Inter- and intra-day
 - System to system (detector to detector)

Anions, Cations, Organic Acids and Bases: Chromatographic Conditions

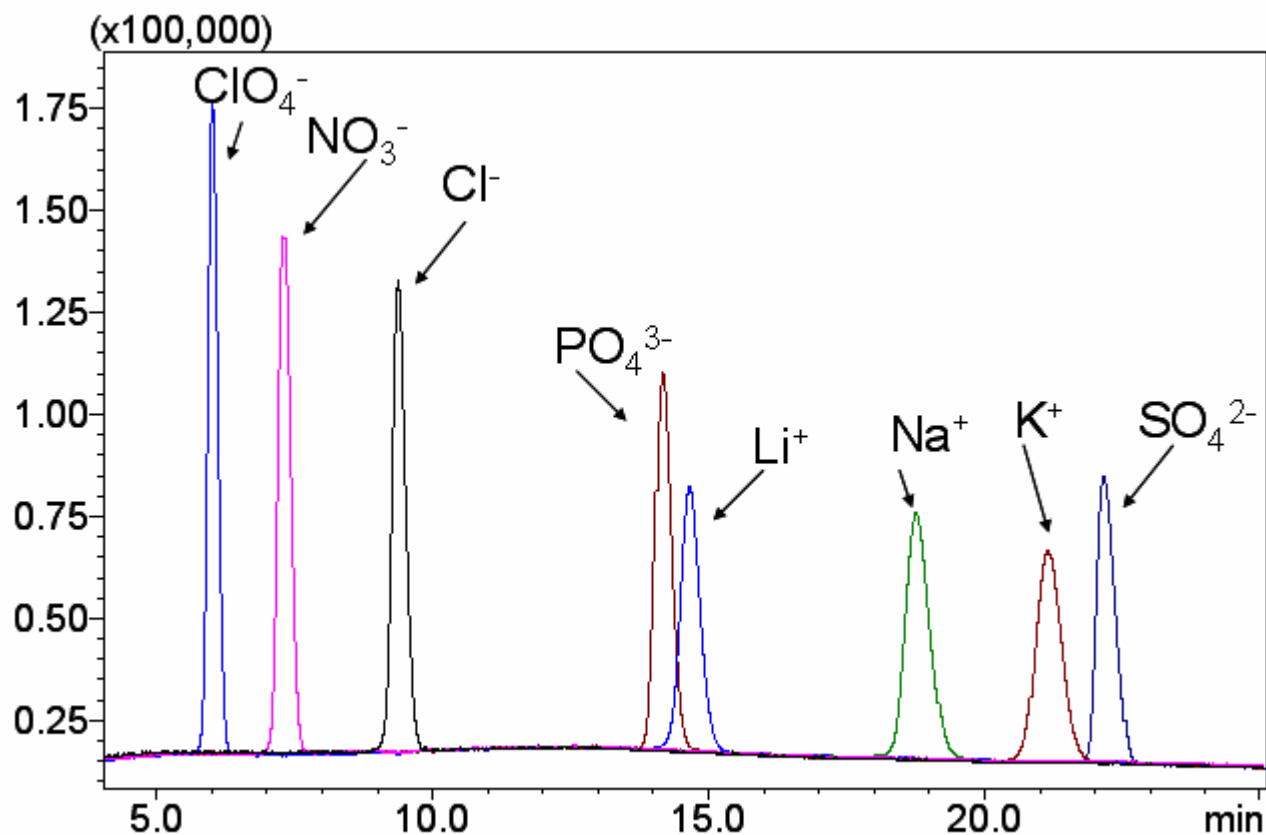
| | |
|--------------------|--|
| Column | Sequant ZIC-pHILIC; 4.6 x 150mm, 5µm (Nest Group) |
| Column Temperature | 30°C |
| Mobile Phase A: | 15% 100mM Ammonium Acetate pH=4.68, 5% Methanol, 20% IPA, 60% Acetonitrile |
| Mobile Phase B: | 50% 30mM Ammonium Acetate pH=4.68, 5% Methanol, 20% IPA, 25% Acetonitrile |
| Flow Rate | 0.5mL/min |
| Injection Volume | 10µL |
| Gradient | Initial: Three minute hold @ 20% B 24 minute linear gradient to 70%B 2 minute hold @ 70%B Return to initial over 10 minutes |
| Corona | 100pA range, no filter |
| Sample Vial | Polypropylene or certified borosilicate |

Note: One Column, One System, One Method and One Detector for all Analyses!!!

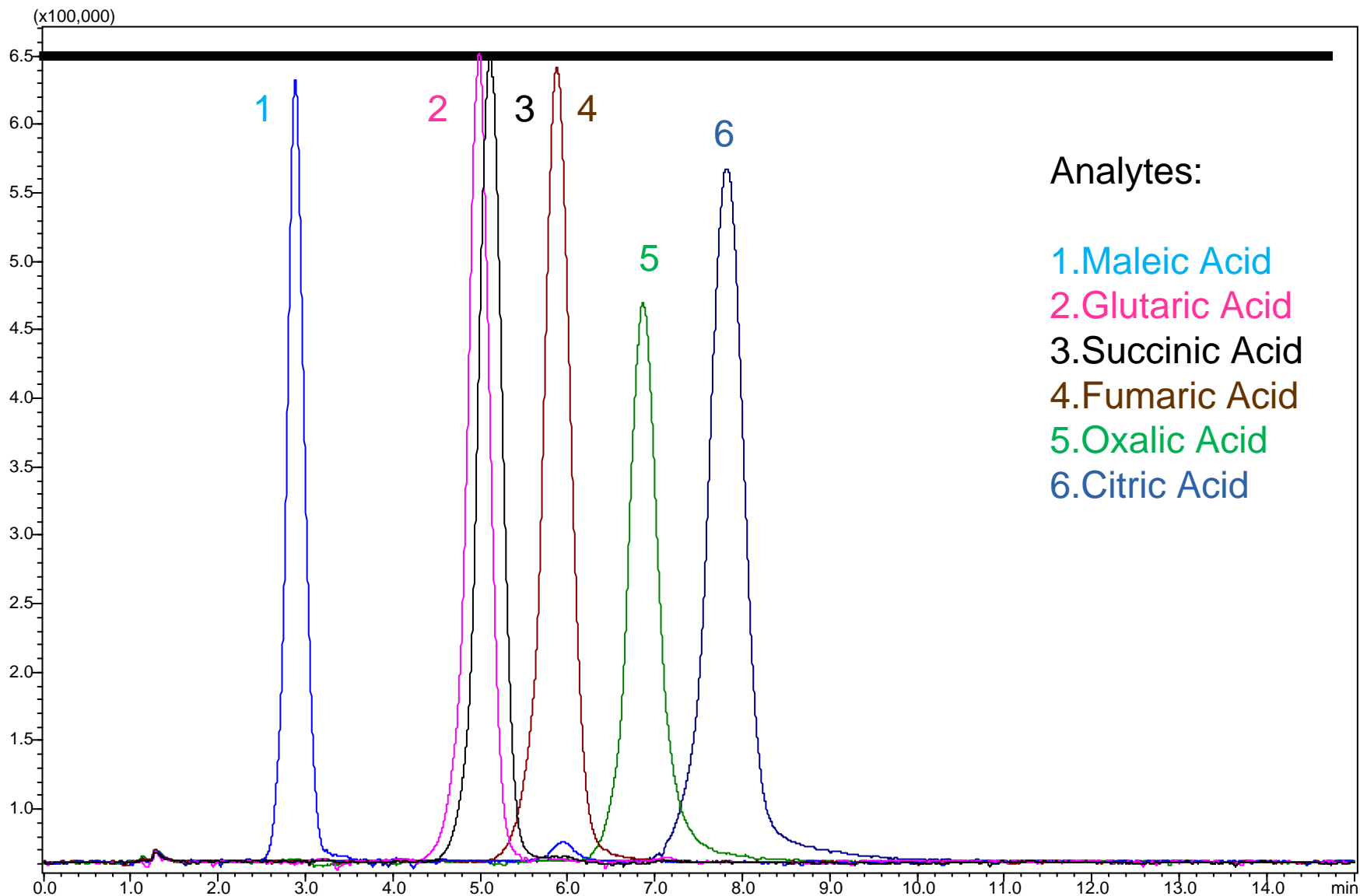
API and Counterion in Single Run



Anions and Cations in Single Run by CAD/HILIC



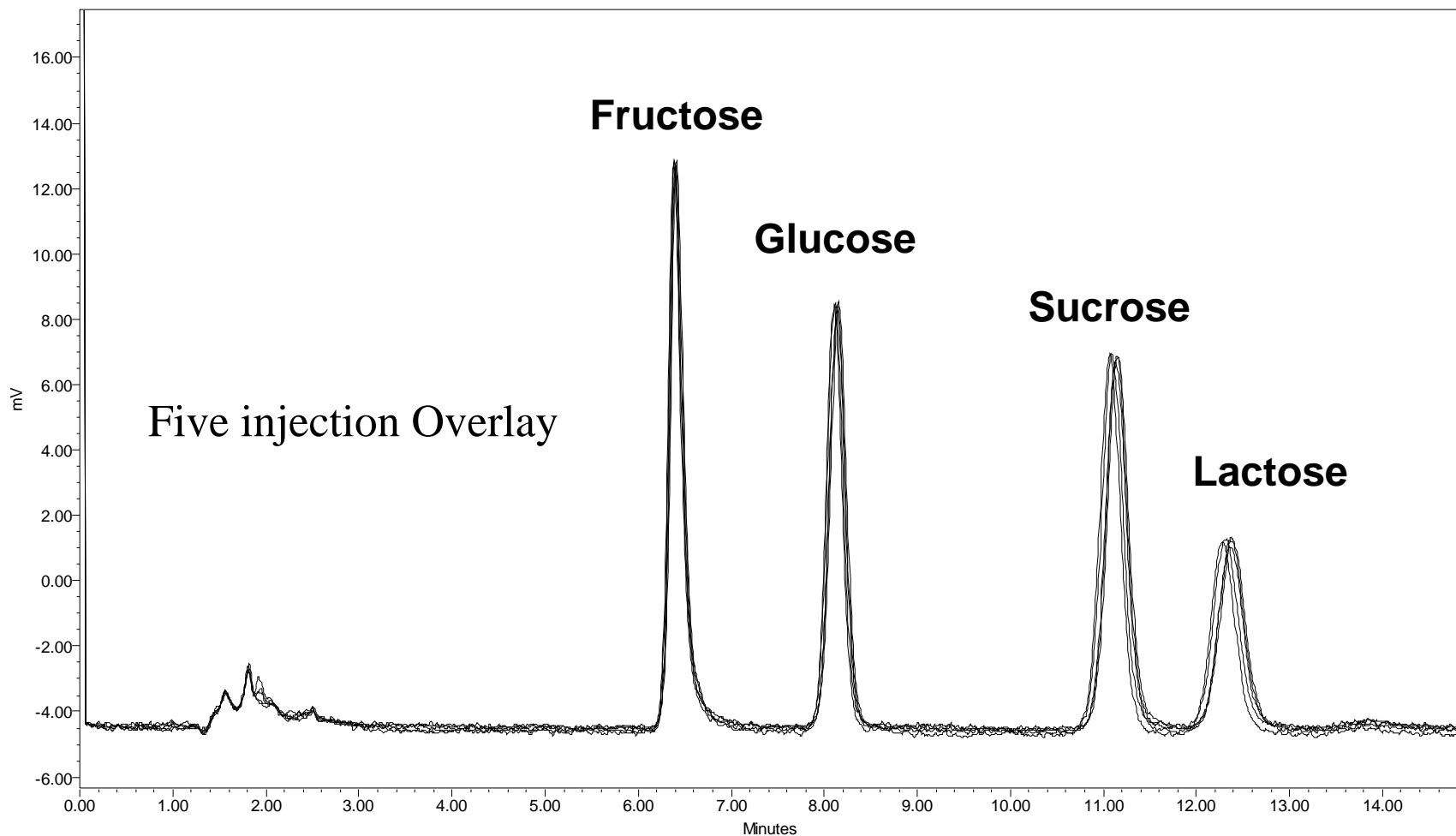
Organic Acids by CAD/HILIC



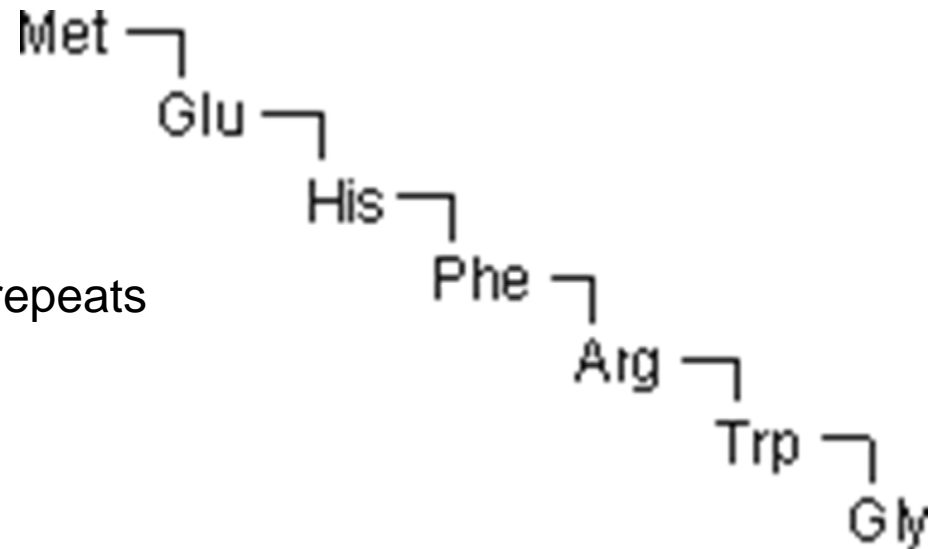
Sugars in Fermentation Broth

- Mobile Phase: 25/75 water/ACN
- Column: Shodex Asahipak NH₂P-50 4.6 x 250mm 5μm
- Column Temperature: 35 °C
- Flow Rate: 1.0 mL/min.
- Sample: 10 μg/mL each in 30/70 water/ACN
- Injection Volume: 10 μL
- CAD: 35 psi Nitrogen flow, 100 pA range, no filter

Sugars by CAD



Peptide Case Study: Adrenocorticotrophic Hormone Fragment 4-10

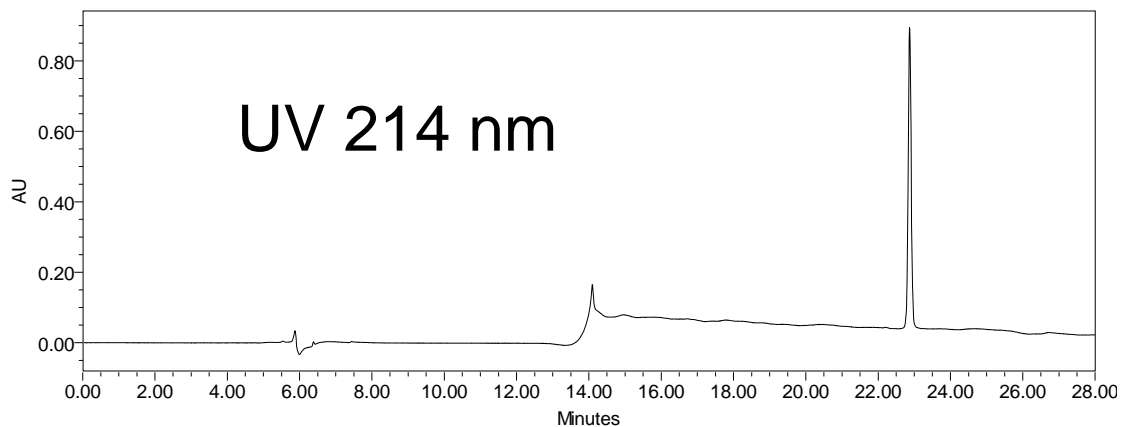
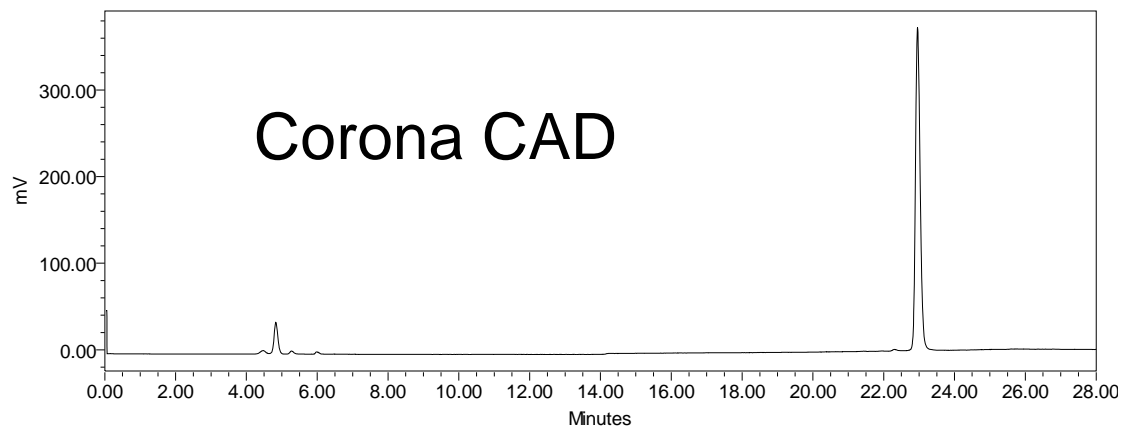


- Hepta-Peptide
 - Seven discreet AA's with no repeats
 - MW 962.1
- Peptide Stability
 - AA or peptide fragment degradants
- Amino Acid Analysis
 - Acid Hydrolysis

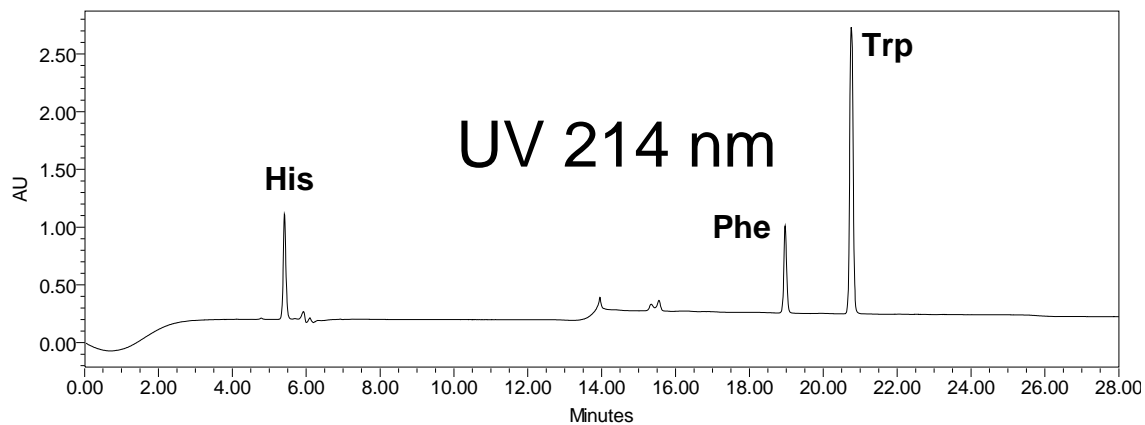
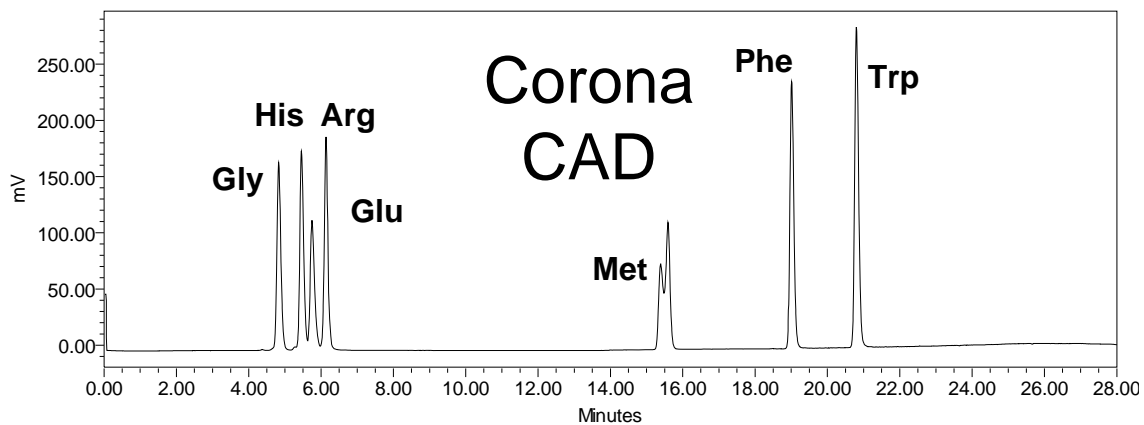
Peptide Separation Conditions

- Mobile Phase: 0.1% TFA in water (A) and ACN (B)
- Column: C18 4.6 x 250mm 5 μ m
- Column Temperature: Ambient
- Flow Rate: 0.6 mL/min.
- Gradient: 5 min hold at 100% A, 0-40% B over 20 min.
- Sample: 1 mg/mL
- Injection Volume: 10 μ L
- CAD: 35 psi Nitrogen flow, 100 pA range, no filter

RP-HPLC Peptide Separation: CAD/UV Detection



RP-HPLC AA Separation: CAD/UV Detection



Summary

- Forced Degradation Study Experimental Design Considerations
 - General and Specific Conditions
- Stability Indicating Method
 - Criteria and Development
- Case Study
 - Optimizing Conditions
 - Column Comparisons



Acknowledgements

- Colleagues @ Synomics
 - Amber Awad
 - John Pirro
 - Debby Hartley

And:



mswartz@synomicspharma.com