Stability of Active Pharmaceutical Ingredients Under High Temperature Liquid Chromatography Conditions

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Outline

- Advantages of high temperature HPLC
- What is temperature programmed HPLC?
- Thermal decomposition of analytes
- DCP
- Thermal decomposition of API’s
- Conclusions

Faster and More Efficient Separations

- **Speed**
  - Flatter van Deemter curves allow operation at flow rates many times optimal velocity
- **Higher efficiency - better resolution**
  - Increased diffusion rates provide lower plate heights at higher flow rates
  - Lower viscosity and back pressure permits higher flow rates with smaller particle size packings

Better Chromatography with Temperature Gradient Programming

- **Change retention through temperature gradient programming**
  - Replace solvent gradients with temperature gradients
  - Water less polar and more like methanol so less organic modifier needed

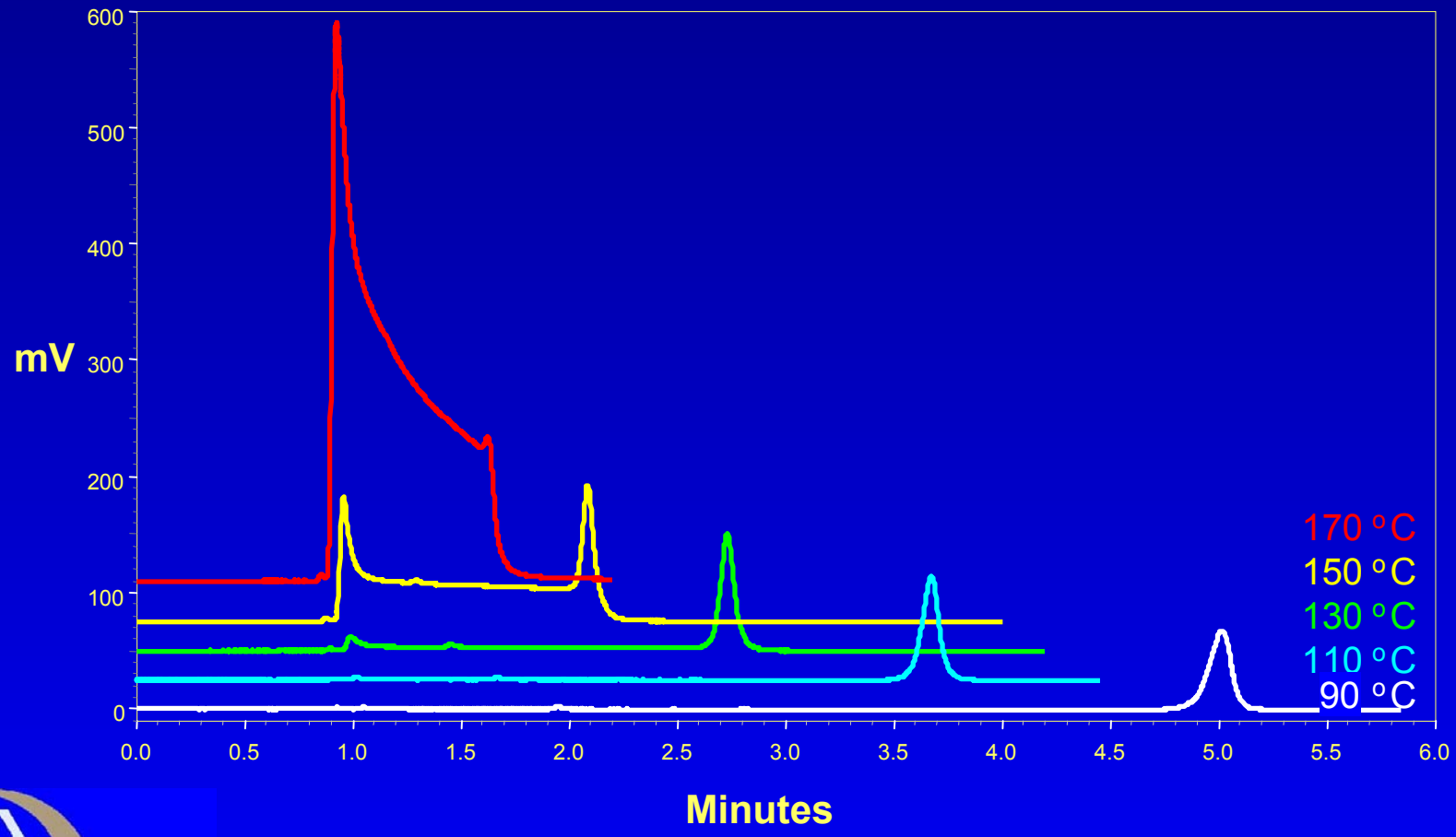
The Selerity Polaratherm Total Temperature Controller

- Forced air oven and chiller
- Isothermal and thermal gradient operation
  - Sub-zero to 200°C
  - Flow rates up to 10.0 mL/min
  - Thermal gradient up to 30°C/min
- Mobile phase preheating
- Peltier effluent cooling
- Vapor sensor
- Compatible with any HPLC system

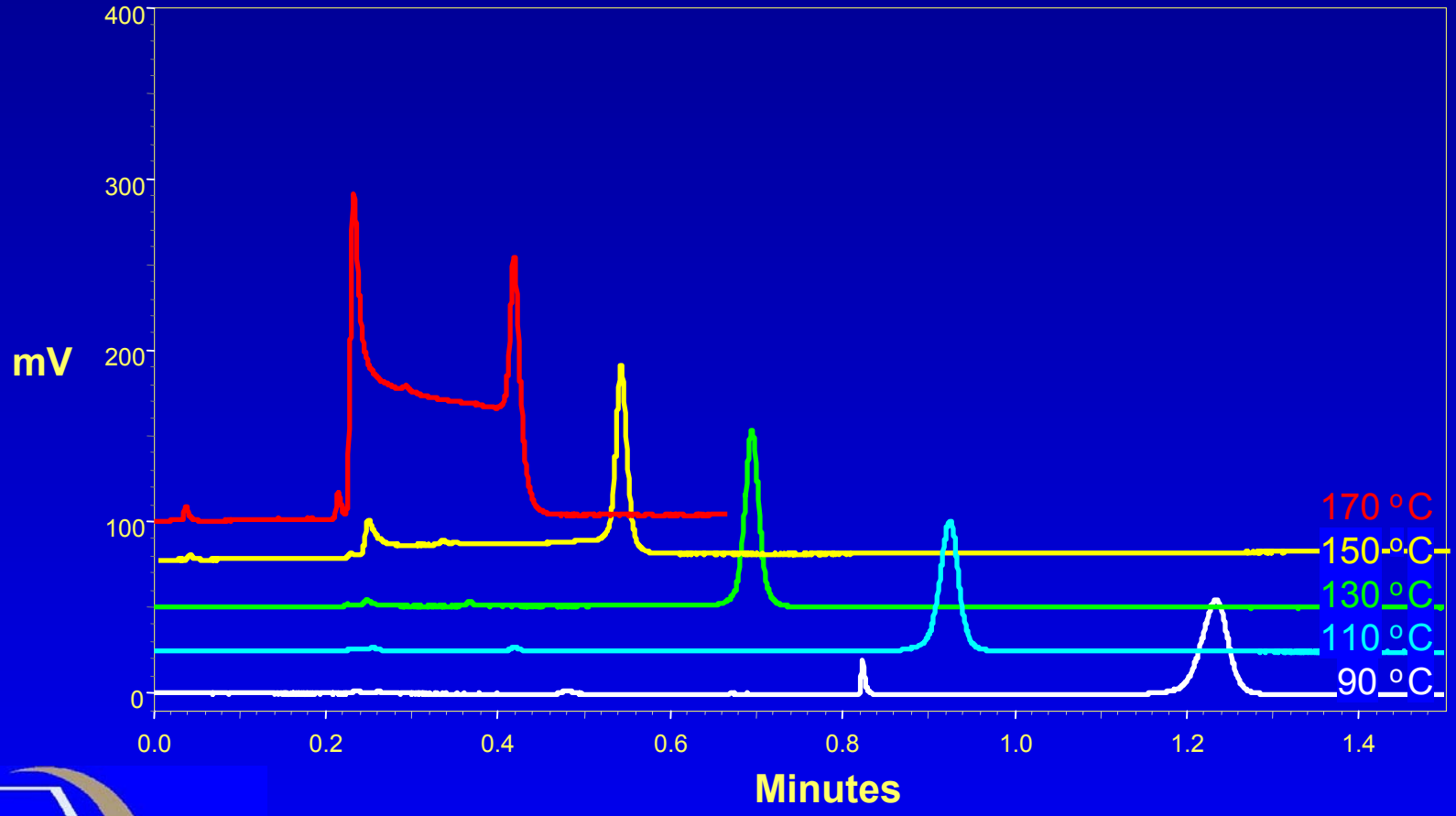
Analysis Conditions for Dicumyl Peroxide

Column:	ZirChrom PBD 100 x 4.6 mm
Mobile Phase:	40:60 acetonitrile:water, isocratic
Detection:	UV@ 254 nm
Injection:	full loop (2.5 uL)
Flow Rates:	1.0, 2.0, 4.0 mL/min
Temperatures:	90 °C, 110 °C, 130 °C, 150 °C, 170°C, isothermal

Dicumyl Peroxide at Five Temperatures at 1.0 mL/min



Dicumyl Peroxide at Five Temperatures at 4.0 mL/min



Normalized Percent of Dicumyl Peroxide Remaining

Temp.	Flow Rate 1.0 mL/min	2.0 mL/min	4.0 mL/min
90 °C	**100%**	**100%**	**100%**
110 °C	**100%**	**99%**	**98%**
130 °C	**88%**	**87%**	**98%**
150 °C	**71%**	**77%**	**77%**
170 °C	**6%**	**23%**	**45%**

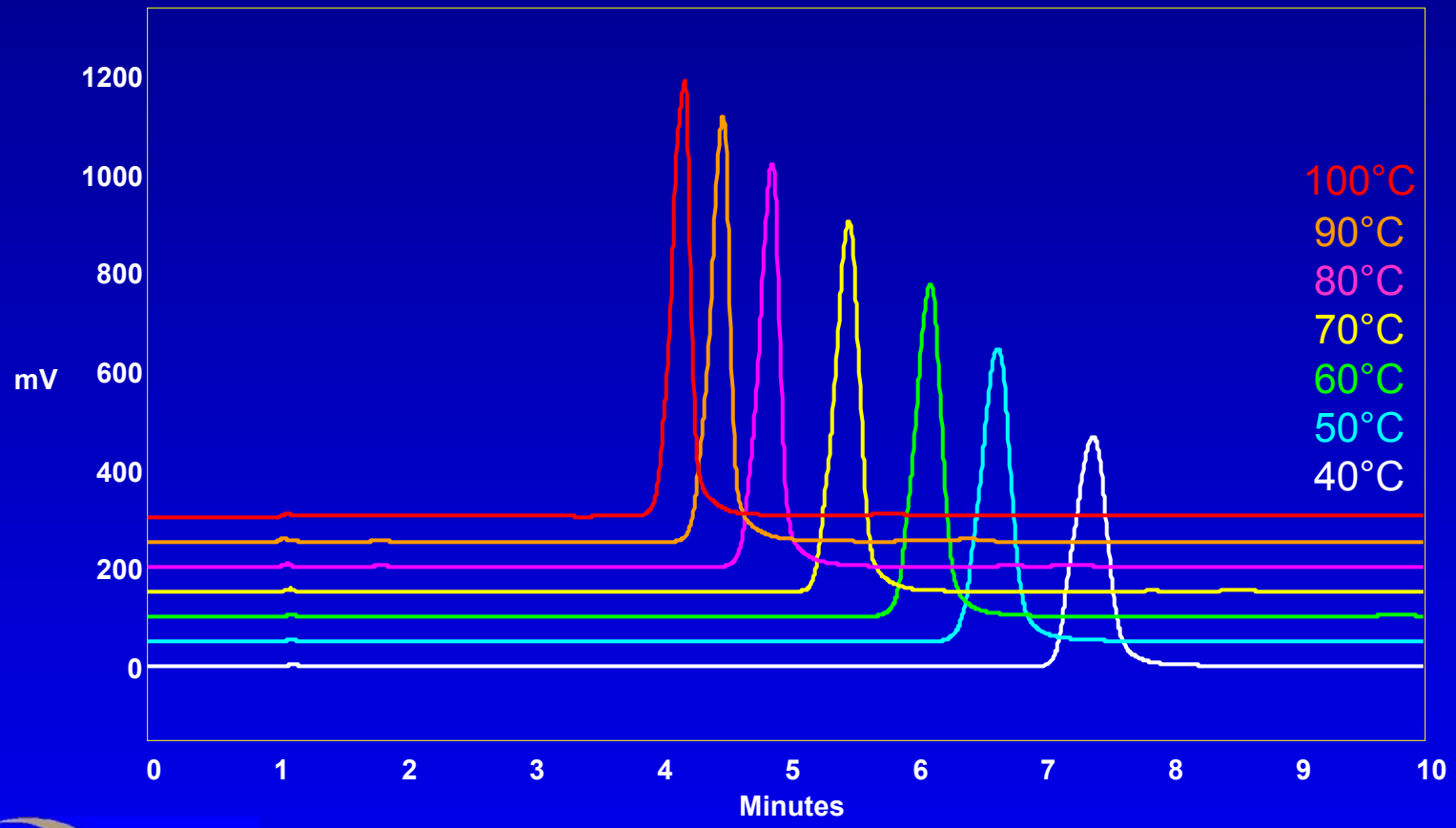
Analysis Conditions for API's at High Temperature

Column:	**Blaze C_8, 3 μm, 150 x 4.6 mm**
Mobile Phase:	**35:65 acetonitrile:water, isocratic**
Detection:	**UV@ 254 nm**
Injection:	**full loop (5.0 uL)**
Flow Rate:	**1.0 mL/min**
Temperatures:	**40°C, 50°C, 60°C, 70°C, 80°C, 90°C and 100°C isothermal**

Analysis of Dexamethasone

- Decadron, Dexameth, Dexone, Hexadrol
- Anti-inflammatory
  - glucocorticoid class of hormones (steroids)
  - same family as cortisone and prednisone
- Recommended storage for API is 2-8°C
- Storage for medication is room temp

Analysis of Dexamethasone



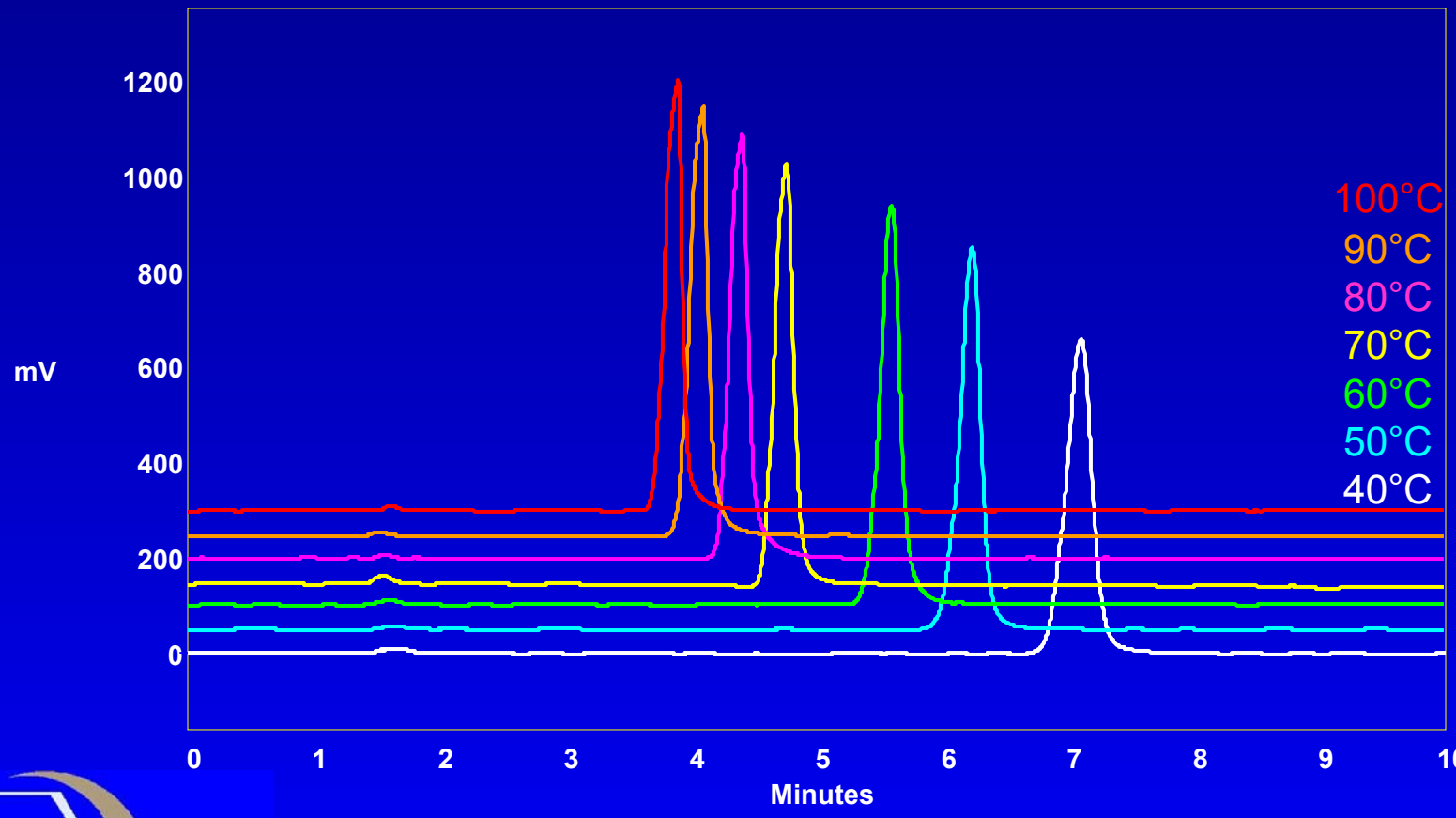
Analysis of Dexamethasone

Temperature	Peak Area
40°C	9,997,556
50°C	10,387,062
60°C	10,777,056
70°C	9,991,160
80°C	9,609,552
90°C	9,911,457
100°C	9,745,998
Mean	**9,987,856**
%RSD	**6.97%**

Analysis of Carbamazepine

- Atretol, Epitol, Tegretol
- Anticonvulsant
  - Used to treat epilepsy/seizures
  - Chewable tablets or liquid suspension
  - Light sensitive
  - Recommended storage for API is 2-8°C
  - Storage for medication is room temp

Analysis of Carbamazepine



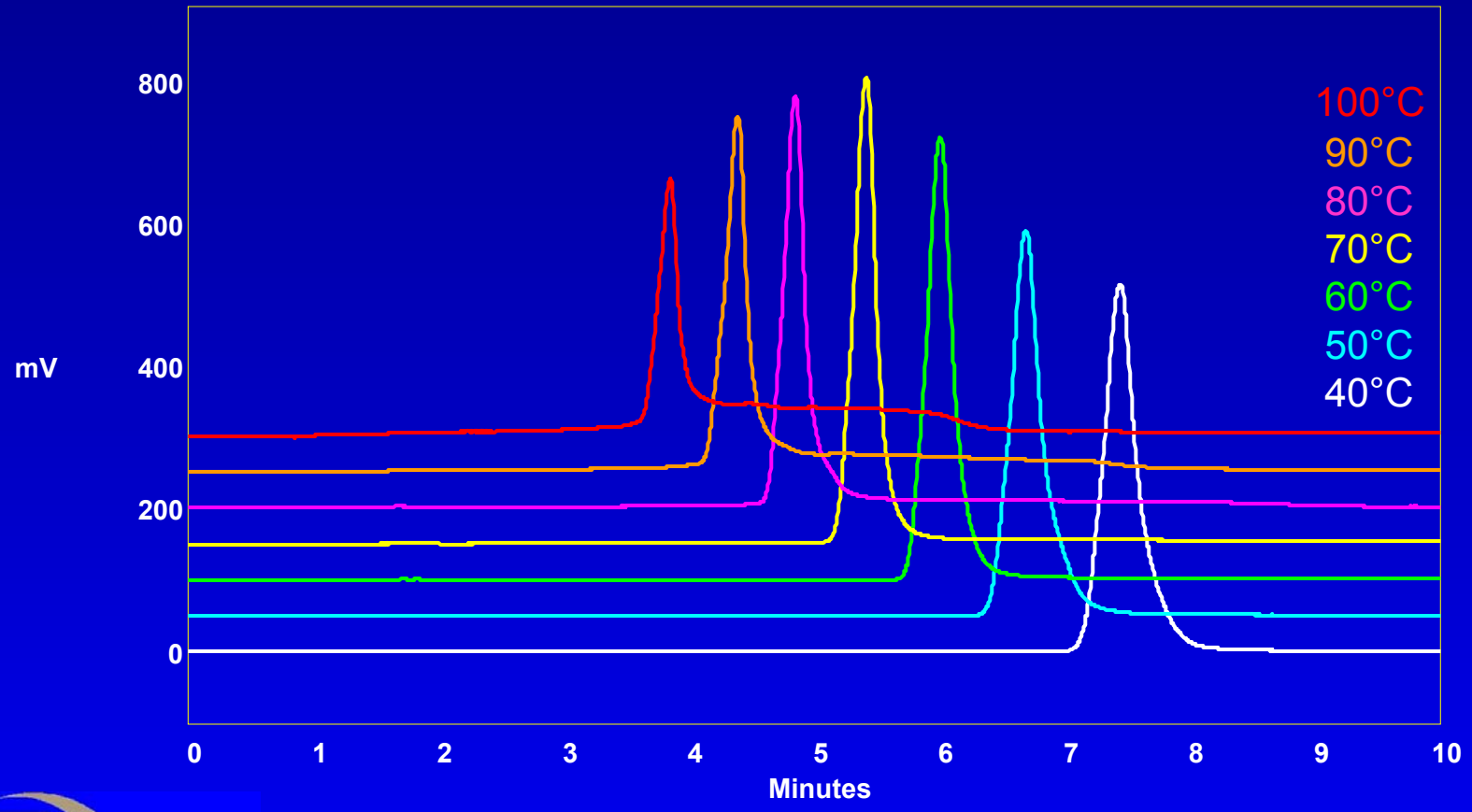
Analysis of Carbamazepine

Temperature	Peak Area
40°C	10,122,934
50°C	10,078,074
60°C	9,941,243
70°C	9,785,801
80°C	9,749,546
90°C	9,348,786
100°C	9,835,455
Mean	**9,837,405**
%RSD	**2.99%**

Analysis of Lansoprazole

- Prevacid
- Inhibits gastric acid secretion
- Melts with decomposition at 166°C
- Degrades in aqueous solution
  - rate of degradation increases with decreasing pH
  - $T_{1/2}$ is approximately 0.5 hour at pH 5.0 and approximately 18 hours at pH 7.0 at 25°C
- Storage temp for API is 2-8°C, room temp for medication

Analysis of Lansoprazole



Analysis of Lansoprazole

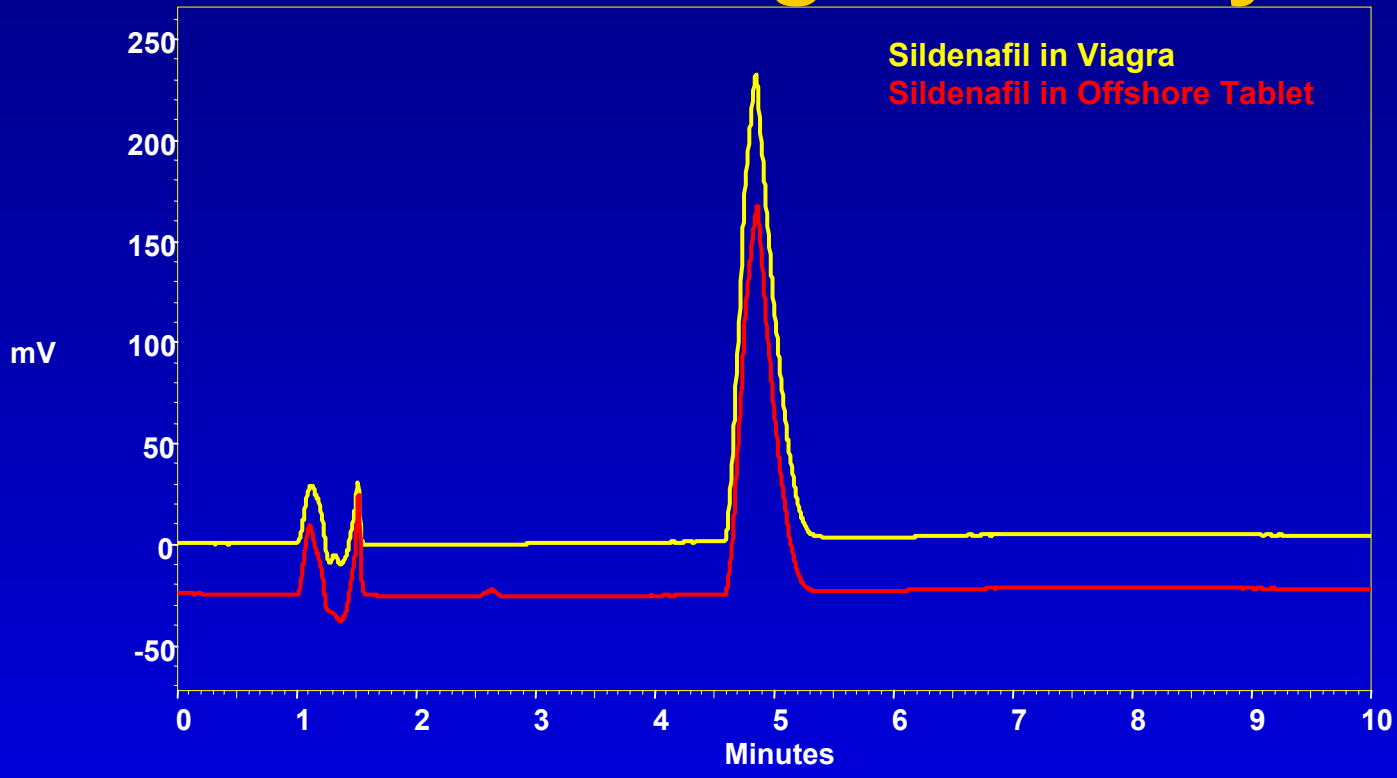
Temperature	Peak Area
40°C	10,507,929
50°C	10,194,359
60°C	9,851,429
70°C	9,070,418
80°C	7,513,678
90°C	5,977,033
100°C	4,082,027

Sample Prep for Sildenafil Analysis in 100 mg Viagra and Offshore Tablet

- Ground tablet in mortar and pestle
- Extracted with 30 mL 50:50 methanol:water by sonicating for 20 minutes
- Centrifuged at 3000 rpm for 15 minutes
- Diluted supernatant to 100 mL with 50:50 methanol:water (rinsed three times)
- Filtered through 0.45μm nylon prior to injection

Tseng and Lin, *J. of Food and Drug Analysis*, **10**, 112-119 (2002)

Analysis of Sildenafil in Viagra and Offshore Tablet Using a Selerity Blaze C8



Column: Selerity Blaze C8 100 x 4.6 mm, 3μm
Mobile Phase: 35:65 acetonitrile:water with 0.1% TFA.
Flow Rate: 1.0 mL/min
Detection: UV 220 nm
Temperature Program: hold at 40°C for two minutes, ramp to 100°C at 15°/min, hold for six min.

Analysis of Sildenafil in Viagra and Offshore Tablet Using a Selerity Blaze C8

- Assume Pfizer Viagra tablet contains 100 mg of Sildenafil
- Compare peak areas
- Correct for amount of tablet used
- Offshore tablet contains about 80 mg of Sildenafil

Conclusions

- Thermal decomposition of analytes is strongly dependent upon flow rate.
- Faster run times at higher temperatures minimizes decomposition because of limited exposure of the analyte to temperature extremes
- Many compounds can be analyzed at high temperatures, but some analytes will decompose.

Turn up the Heat!

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