

Determination of vitamin A in feeds using HPLC with pyrene internal standard and overnight shake extraction

Huahua Lin, Ludmila Krzton (retired),
Robert L. Smallidge(retired), Ken L. Riter (Nestle-
Purina) and Victoria S. Siegel



Office of Indiana State Chemist

The logo features the letters 'OSC' in a bold, green, sans-serif font. A black test tube with a white stopper is positioned vertically, overlapping the 'O' and 'S'.

OSC Background

Current AOAC Official Method of Analysis for vitamin A is Carr-Price colorimetric method

- Uses corrosive and highly toxic chemical reagents - antimony trichloride, chloroform
- Low-throughput (~ 10 samples per run)
- Time consuming – Saponification, hexane partition, wash hexane twice, hexane evaporation and reconstitution, colorimetric determination



HPLC Method

- **Advantages**
 - ✓ Elimination of hazardous reagents
 - ✓ High sample throughput (≤ 30 samples per run)
- **Procedure**
 - ✓ **Extraction**
 - ▶ 5-10 g feed into 250 mL amber HDPE bottle
 - ▶ Add 20 mL KOH, swirl
 - ▶ Immediately add 80 mL ethanol w/pyrogallol
 - ▶ Accurately add pyrene internal standard
 - ▶ Shake for 16 hours at room temperature

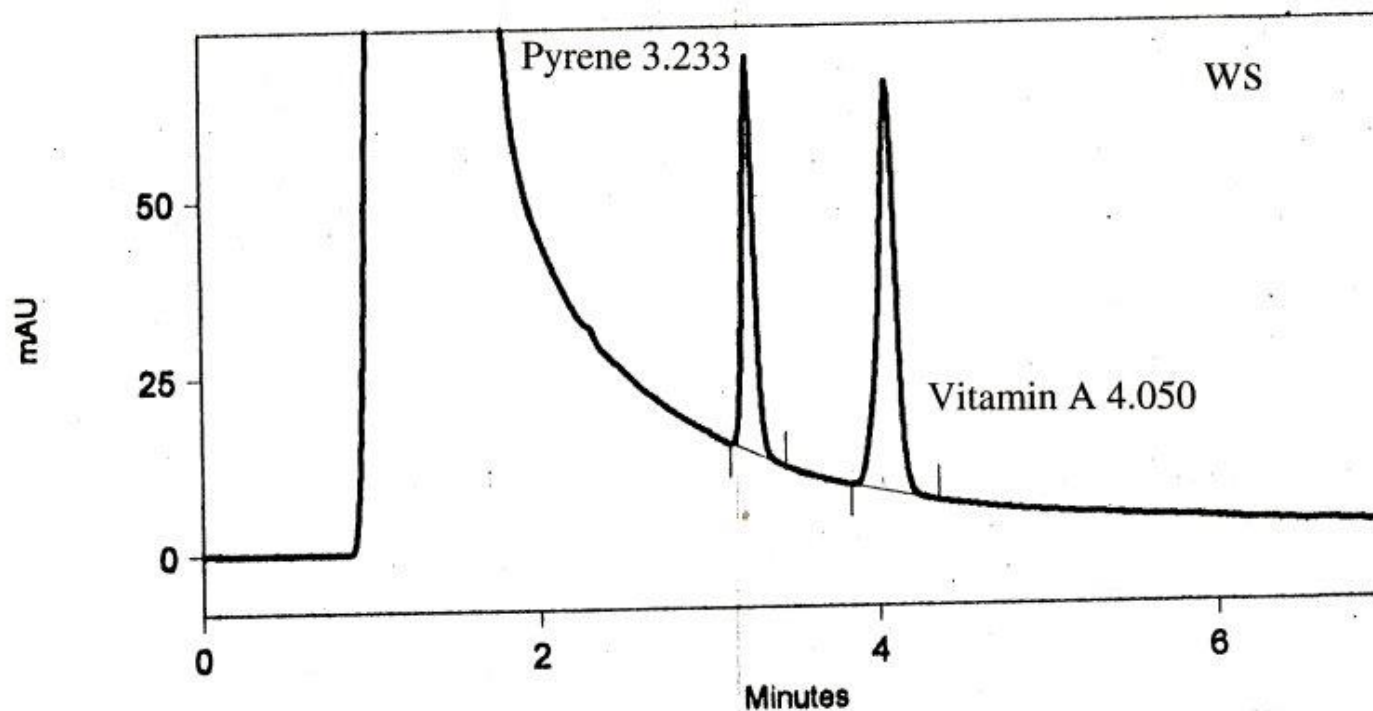


HPLC Method

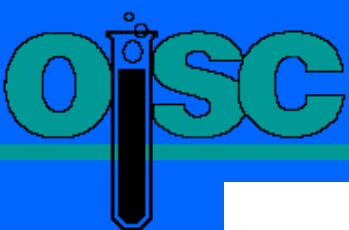
- **Procedure (continued)**
 - ✓ **Neutralization and Dilution**
 - ▶ Add acetic acid in acetonitrile to partially neutralize saponicate
 - ▶ Transfer 3 mL neutralized saponicate to 15 mL centrifuge tube
 - ▶ Add 2 mL acetonitrile to precipitate fatty acid salts
 - ✓ **Determination**
 - ▶ Filter and inject extract on HPLC



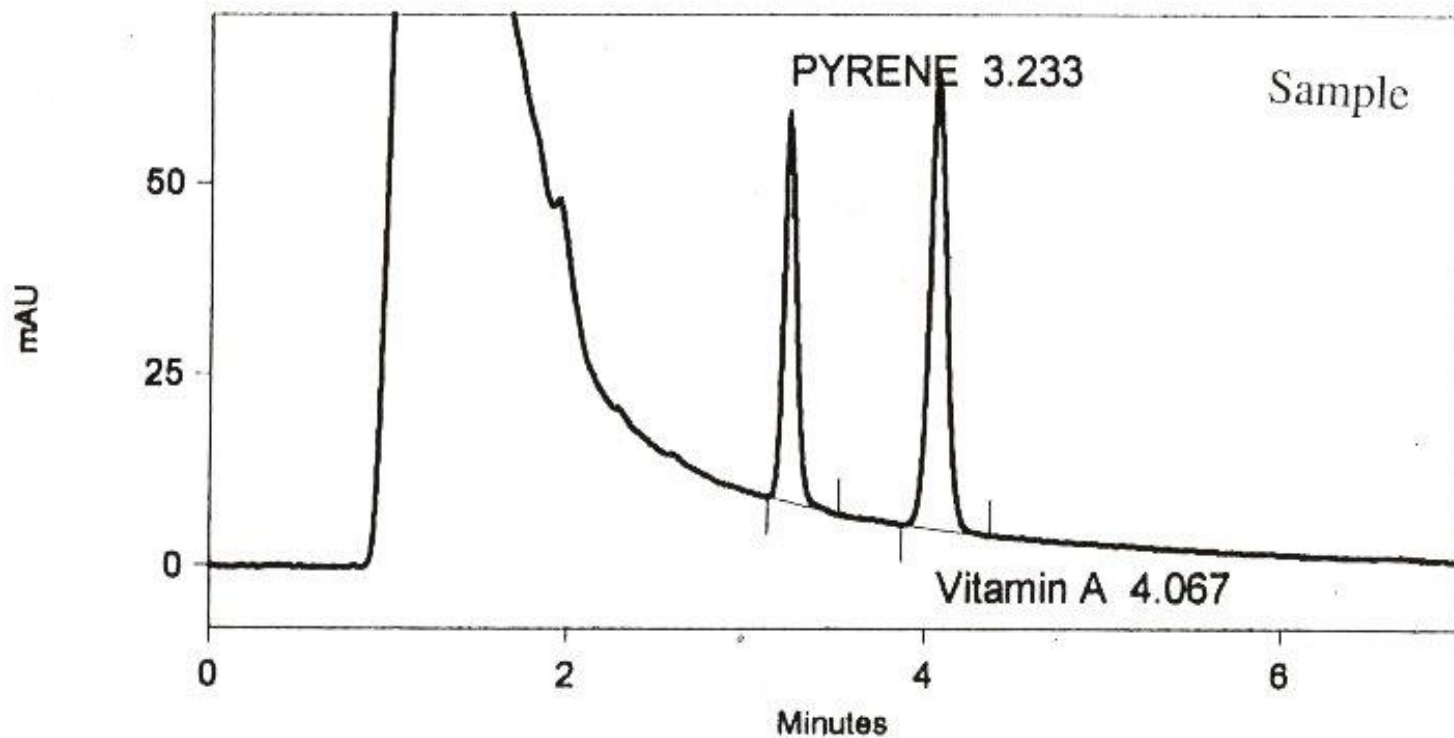
Working Standard



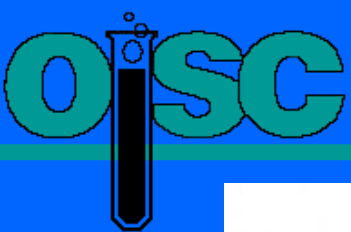
Column: Zobax SB-C18, 150 x 4.6 mm, 3.5 μ m
Mobile Phase: Methanol: Water (95%: 5%)



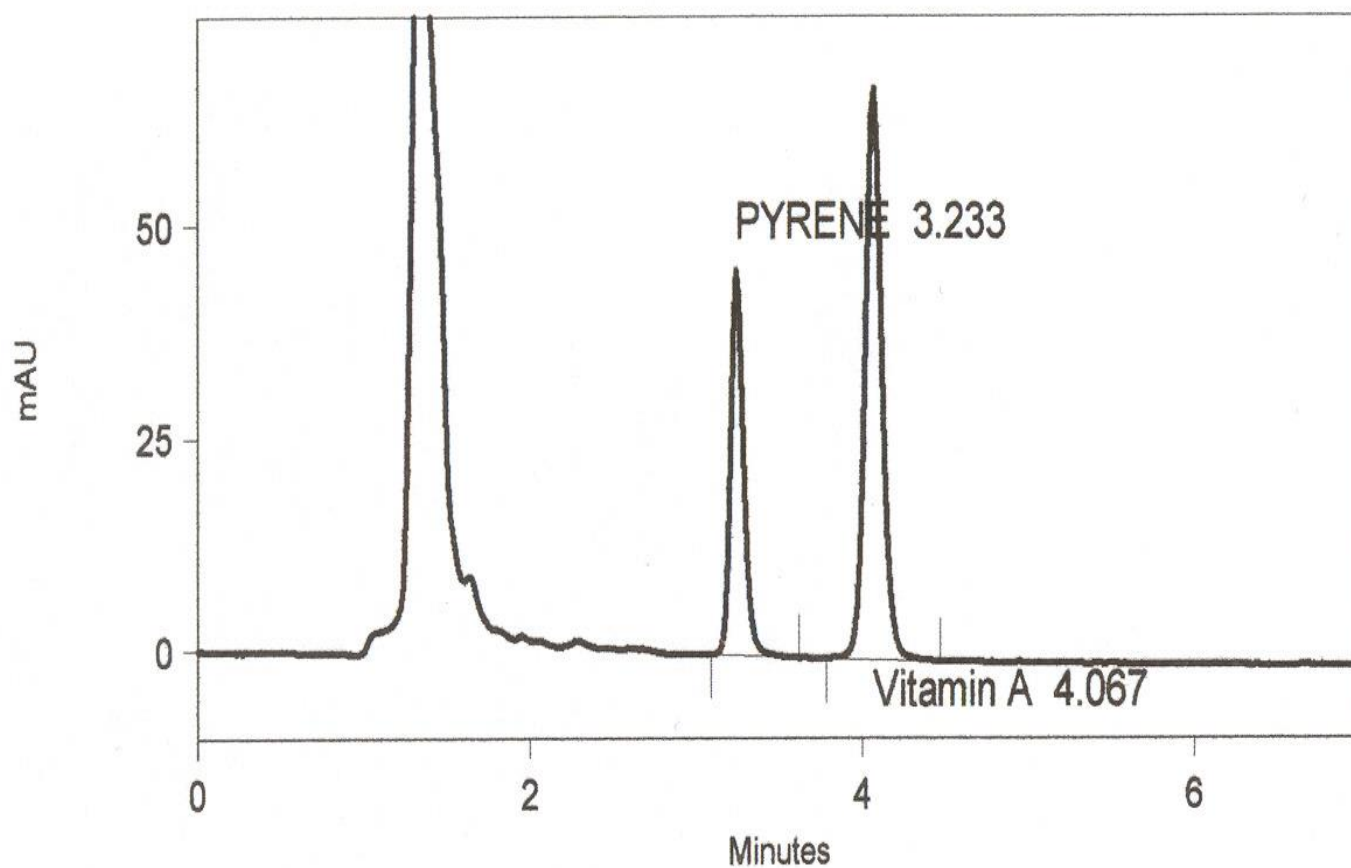
Sample



Column: Zobax SB-C18, 150 x 4.6 mm, 3.5 μ m
Mobile Phase: Methanol: Water (95%: 5%)



Sample





Injection Precision

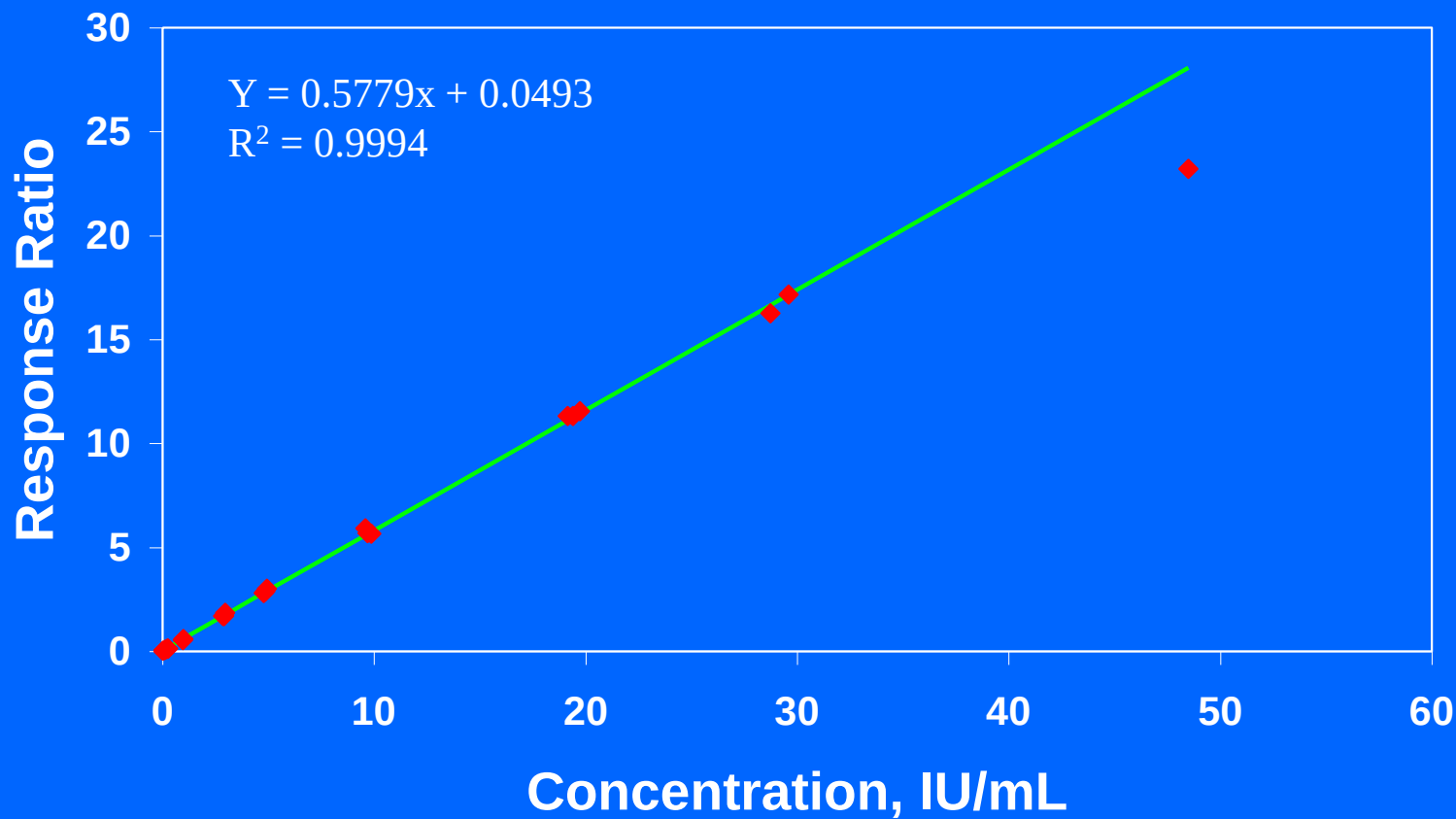
Working Standard Injections

- Beginning of Run
 $n = 5, CV = 0.47$
- Throughout Run
 $n = 20, CV = 0.79$



Standard Curve

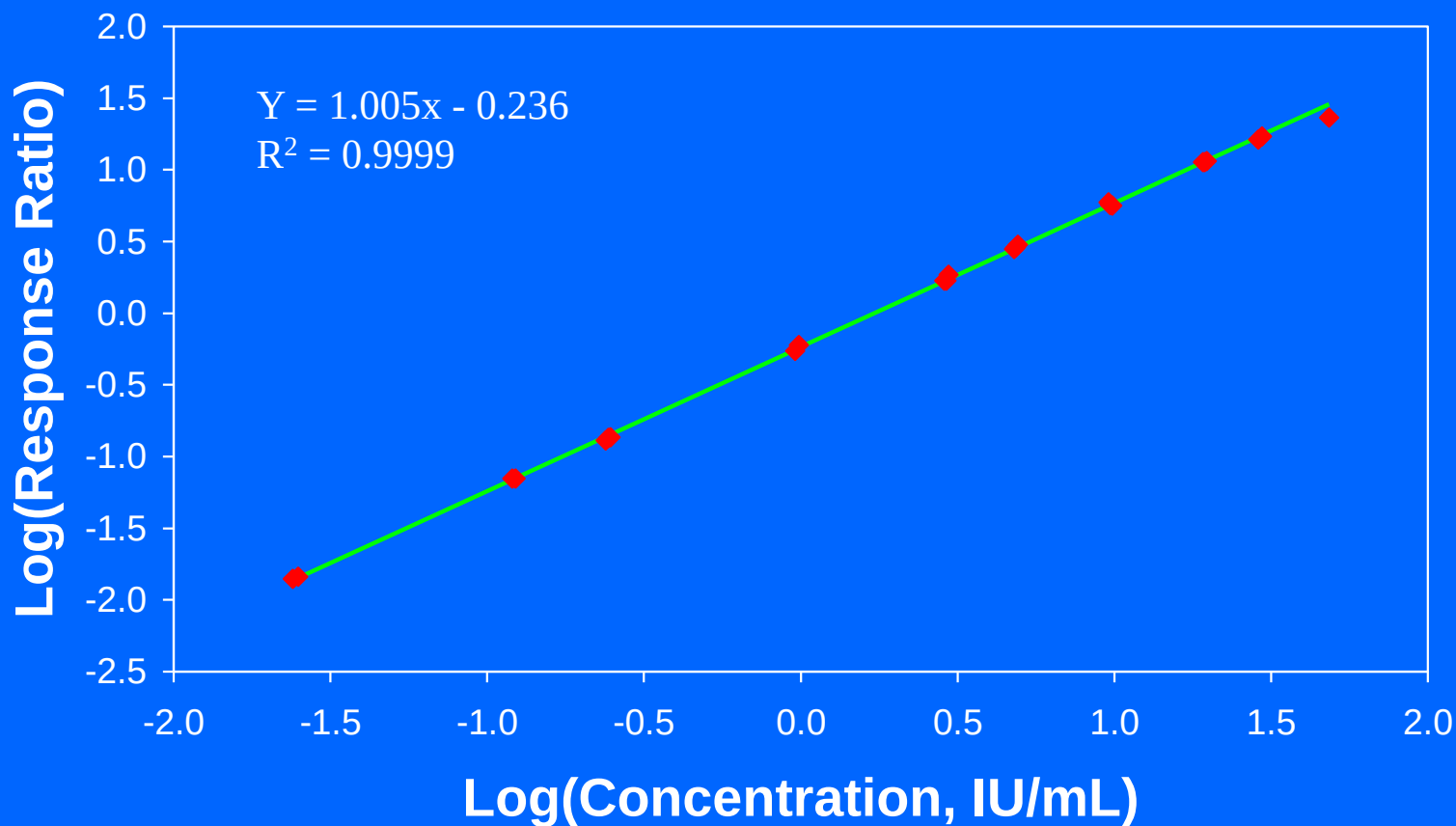
Standard Curve





Log-Log Standard Curve

Log-Log Standard Curve





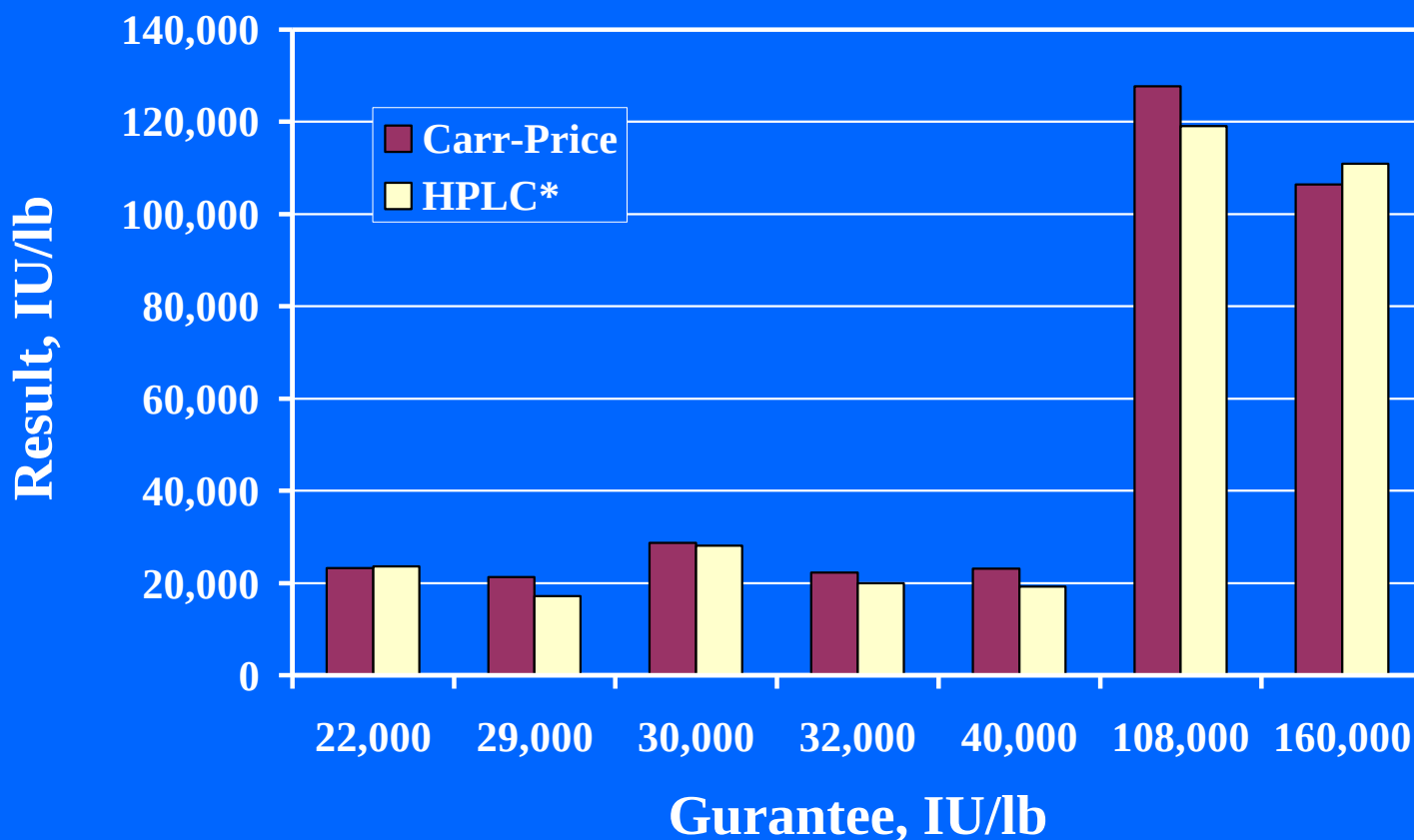
Comparison of HPLC & Carr-Price

	Carr-price Method	OISC HPLC Method
Extraction	Hot Saponification	Overnight Shake
Measurement	Spectrophotometric	HPLC



Colorimetric vs. HPLC, Same Extract

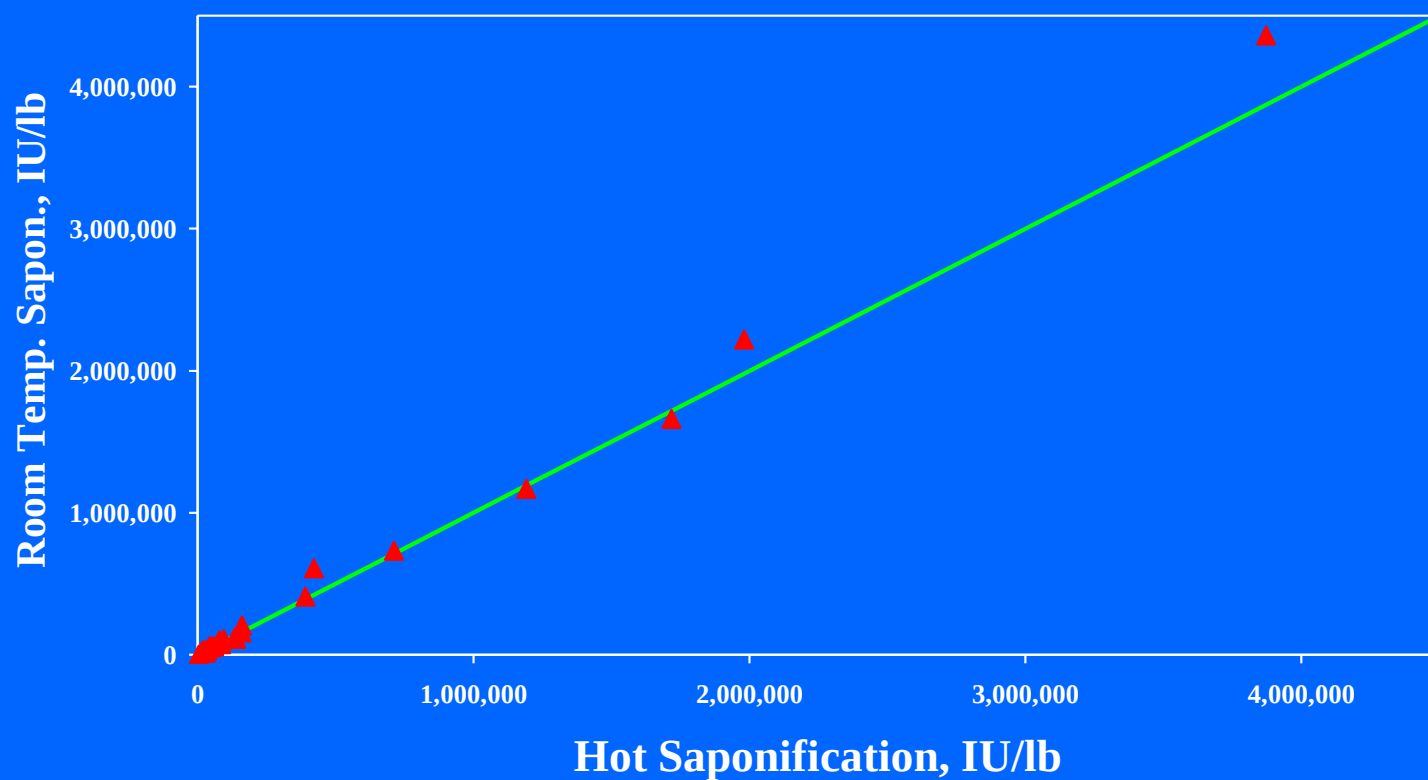
Colorimetric vs. HPLC on Same Extract





Room Temp. vs. Hot Saponification

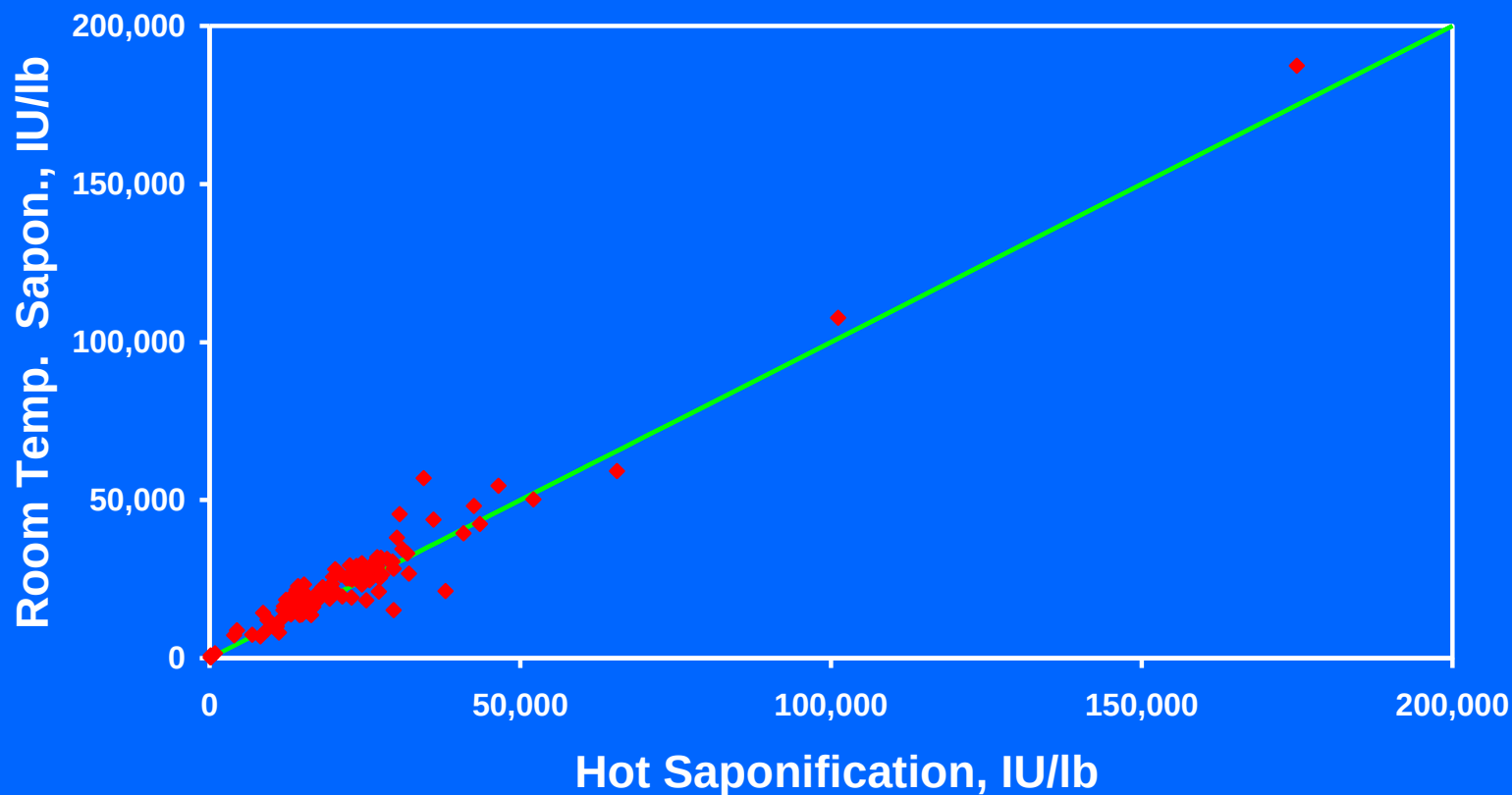
"Meal" Samples





Room Temp. vs. Hot Saponification

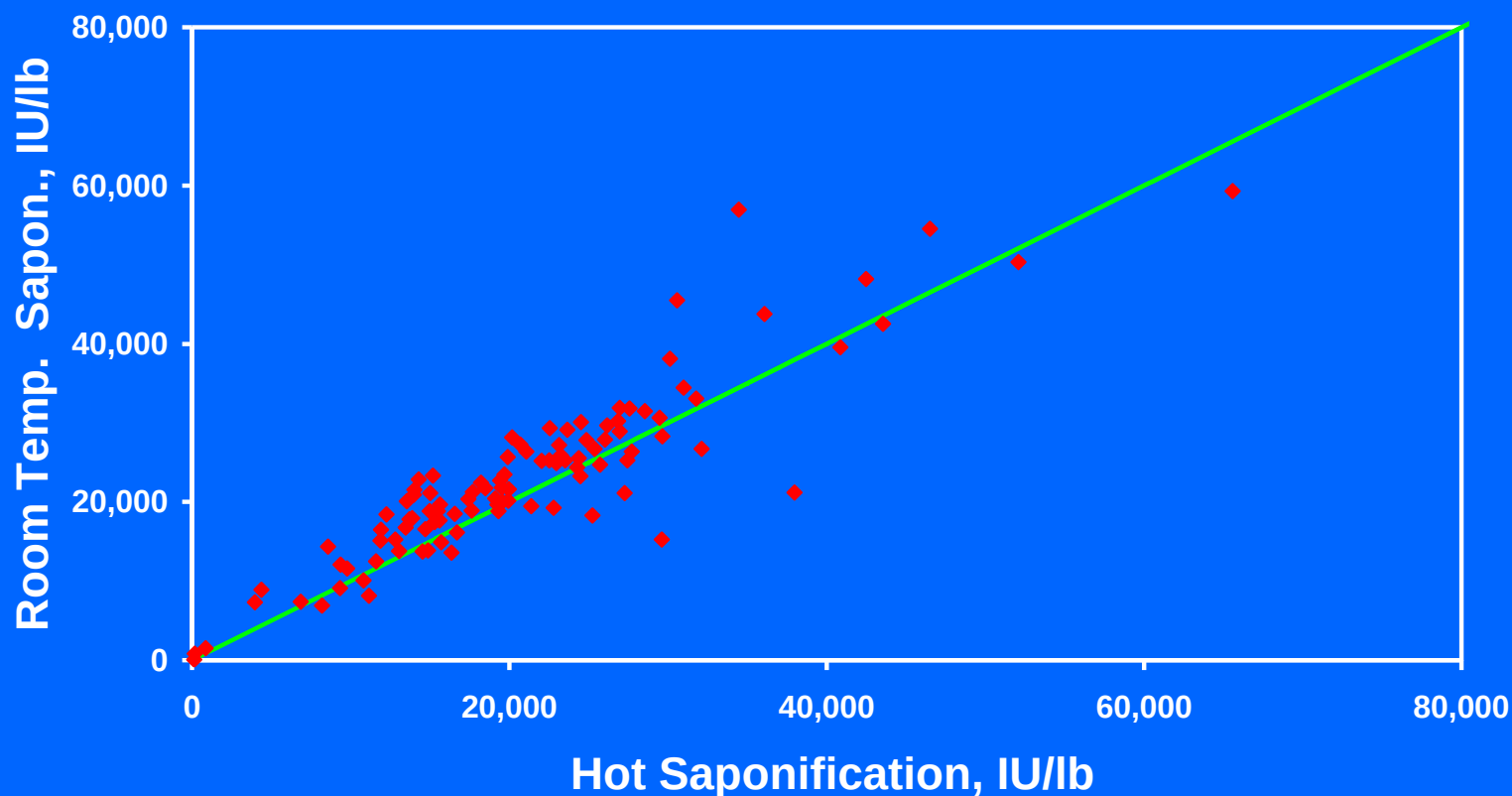
Pelleted Samples





Room Temp. vs. Hot Saponification

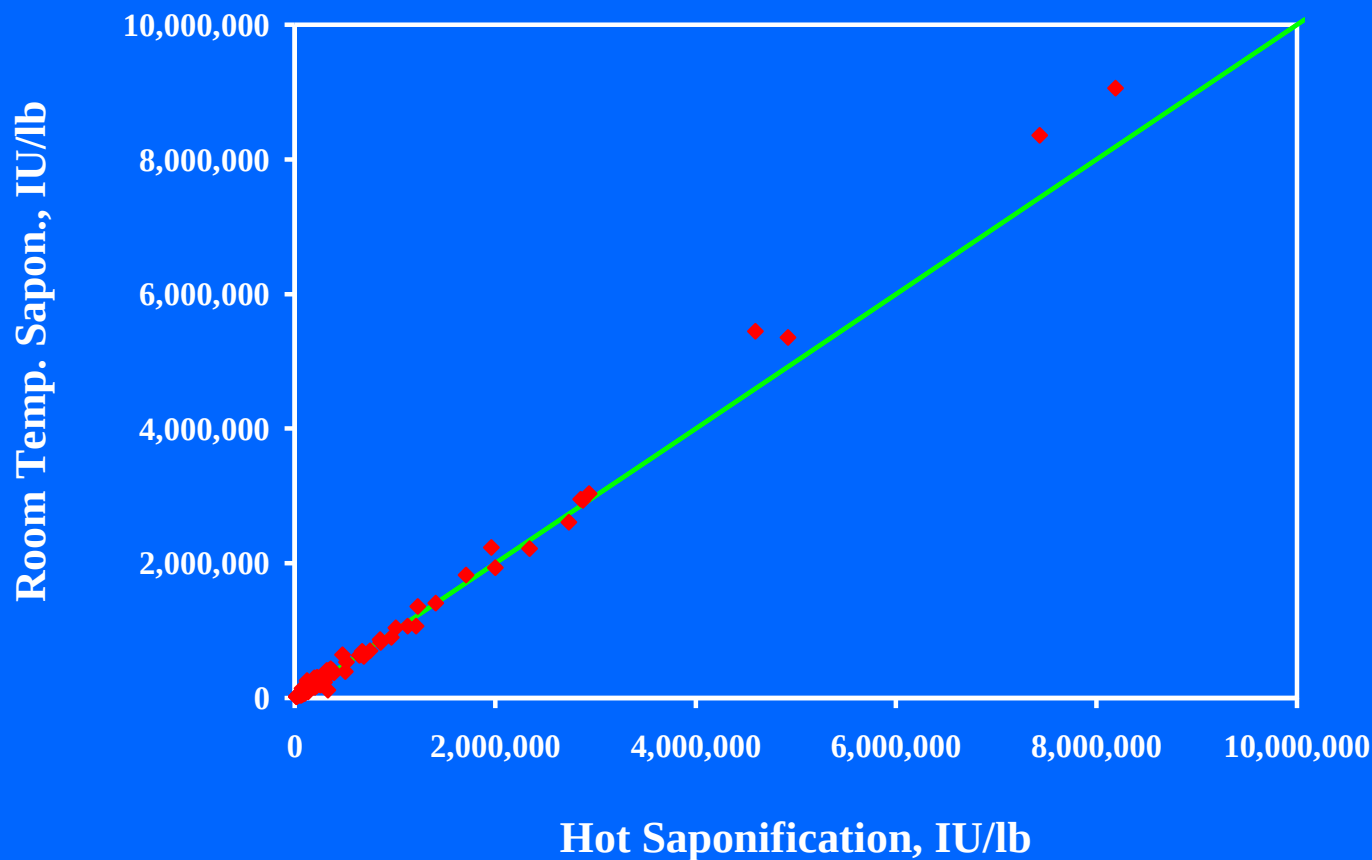
Pelleted Samples, Lower Guarantees





Room Temp. vs. Hot Saponification

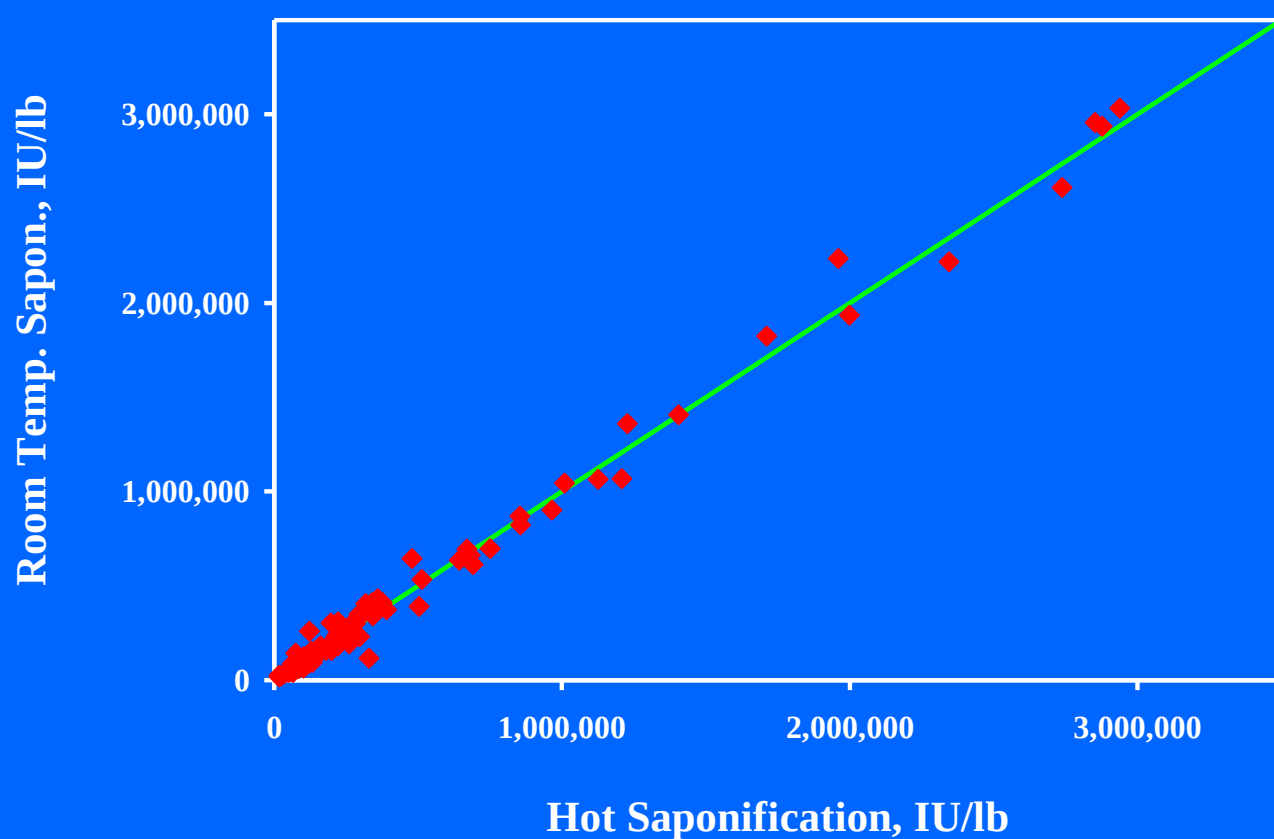
Mineral Samples





Room Temp. vs. Hot Saponification

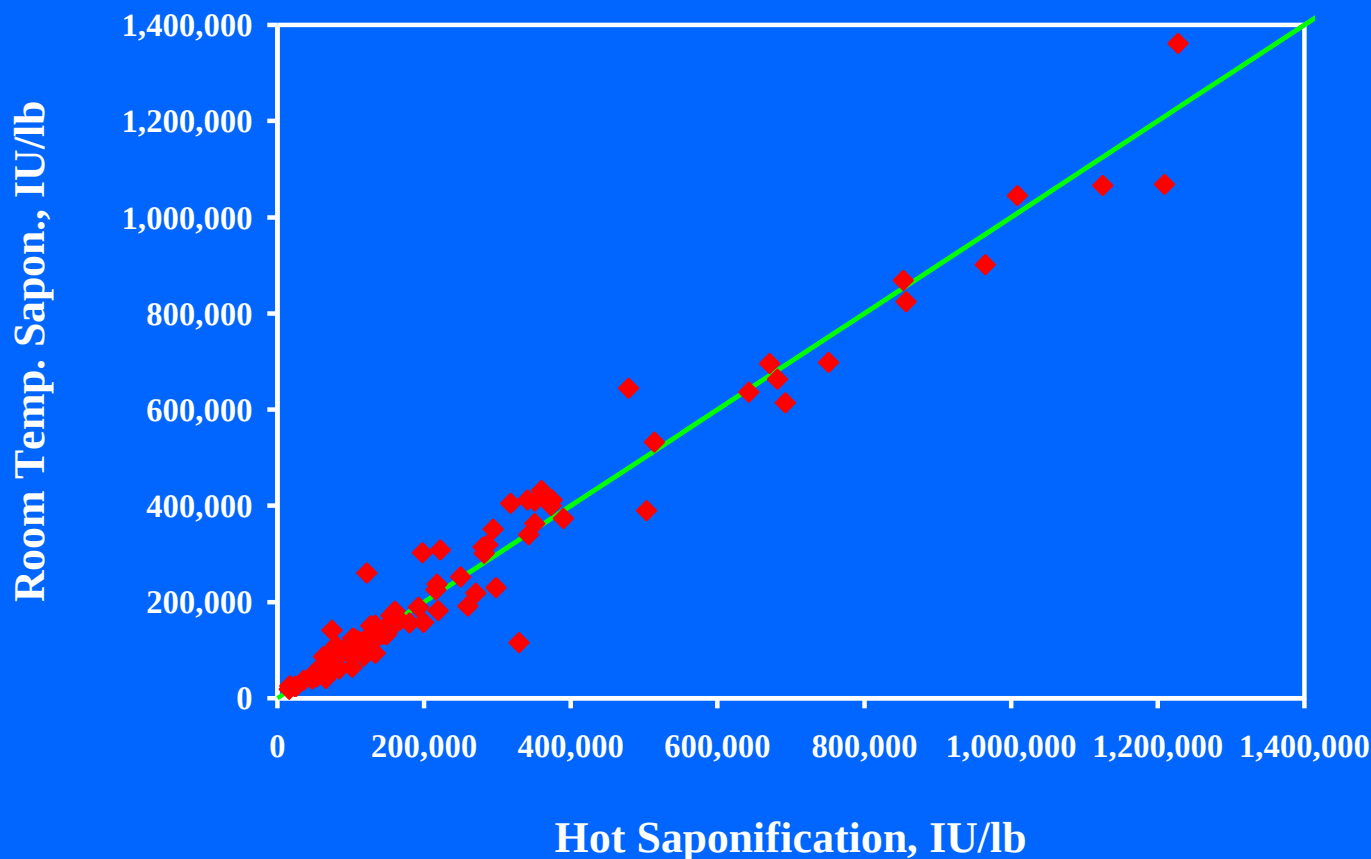
Mineral Samples, Lower Guarantees





Room Temp. vs. Hot Saponification

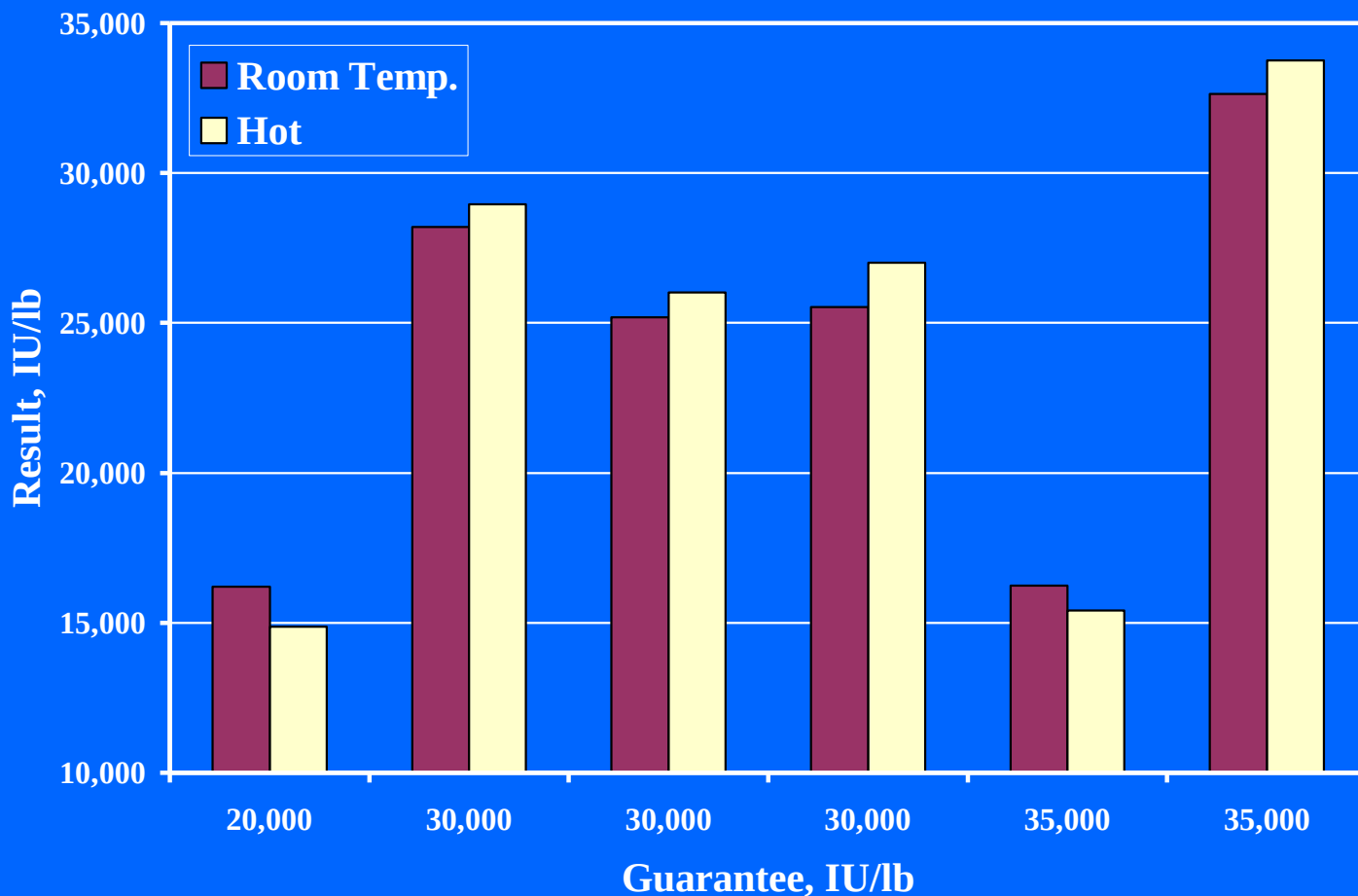
Mineral Samples, Lowest Guarantees





Room Temp. vs. Hot Saponification

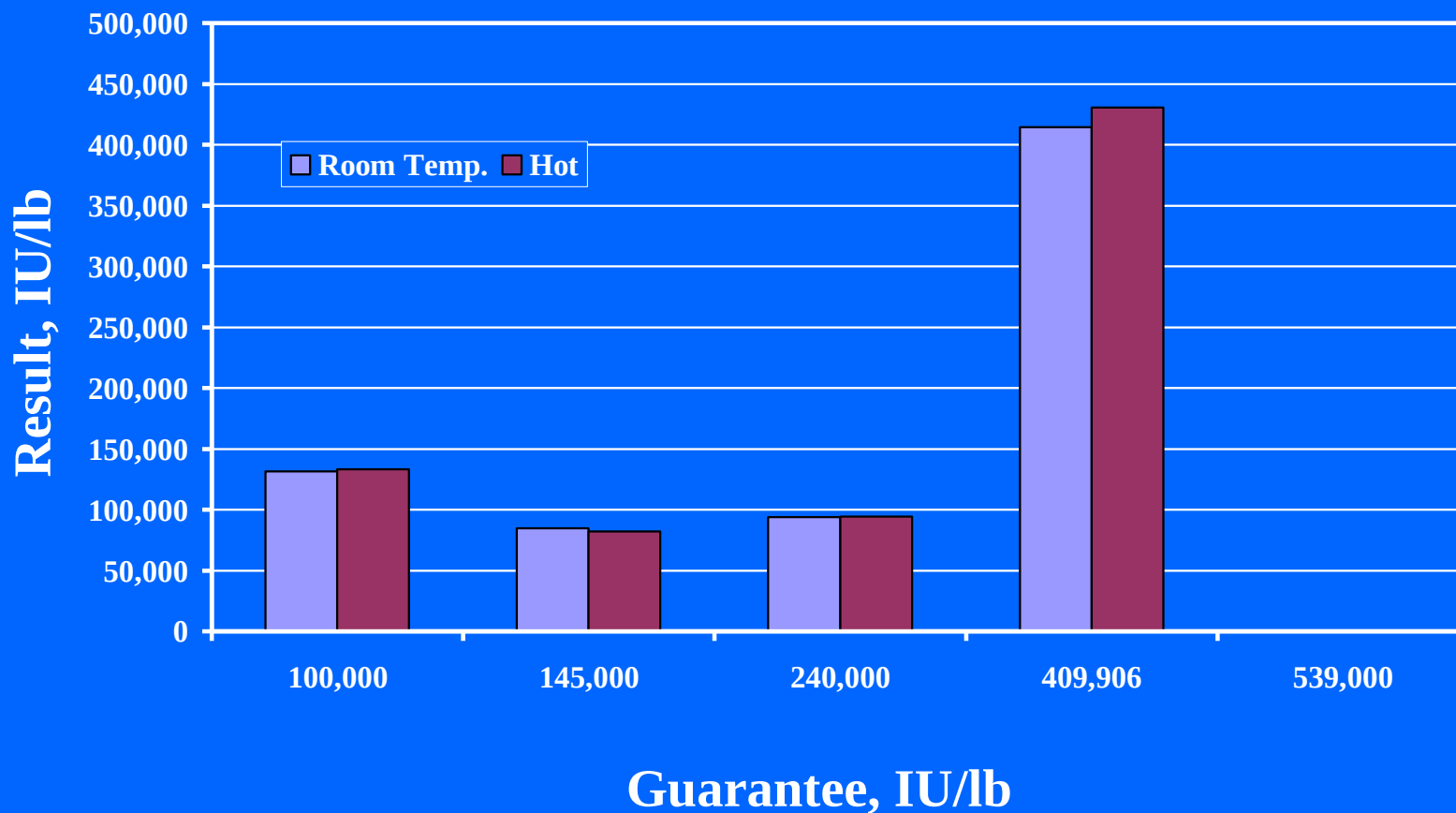
Dry Milk Replacer Samples





Room Temp. vs. Hot Saponification

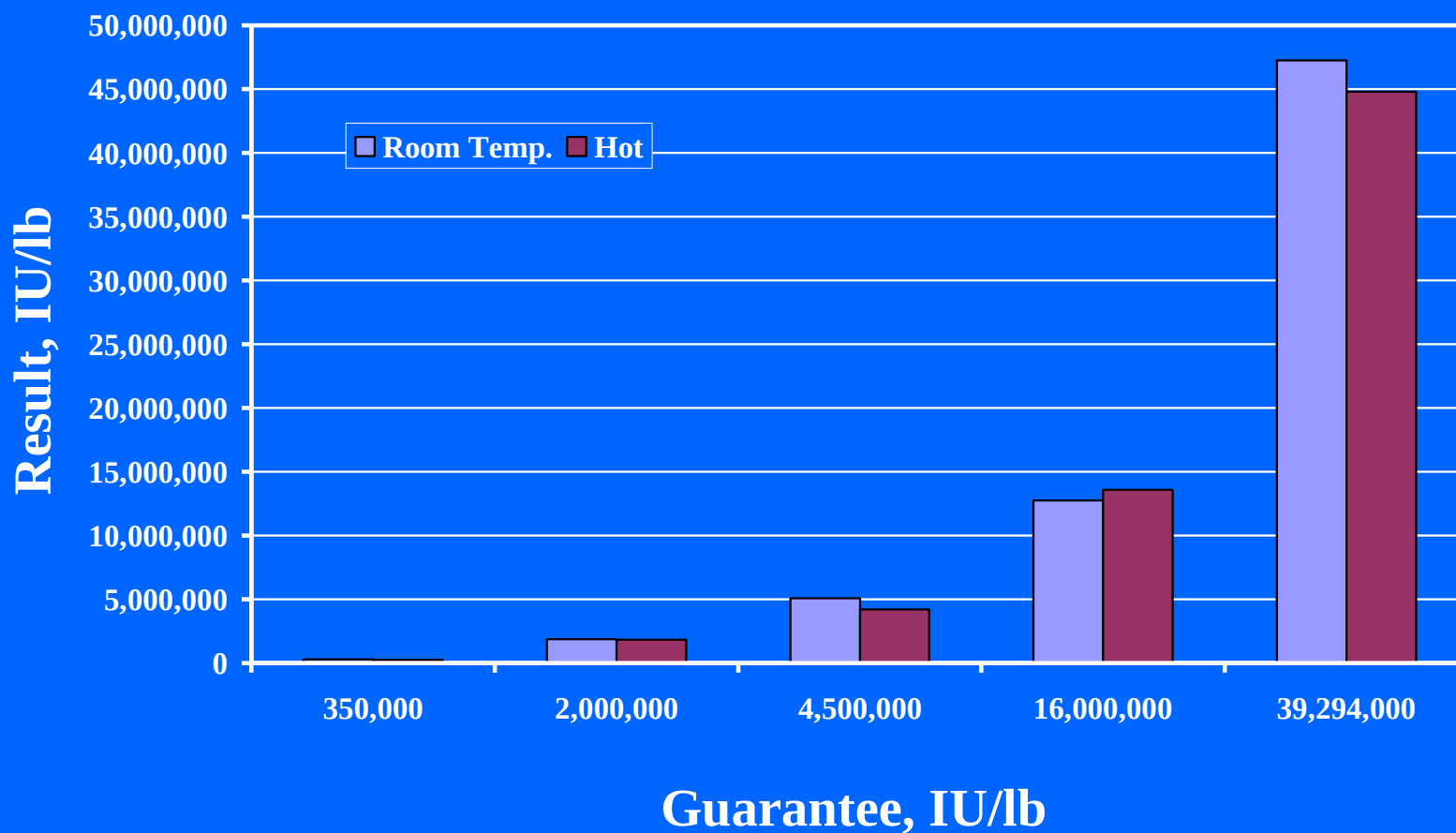
Liquid Samples





Room Temp. vs. Hot Saponification

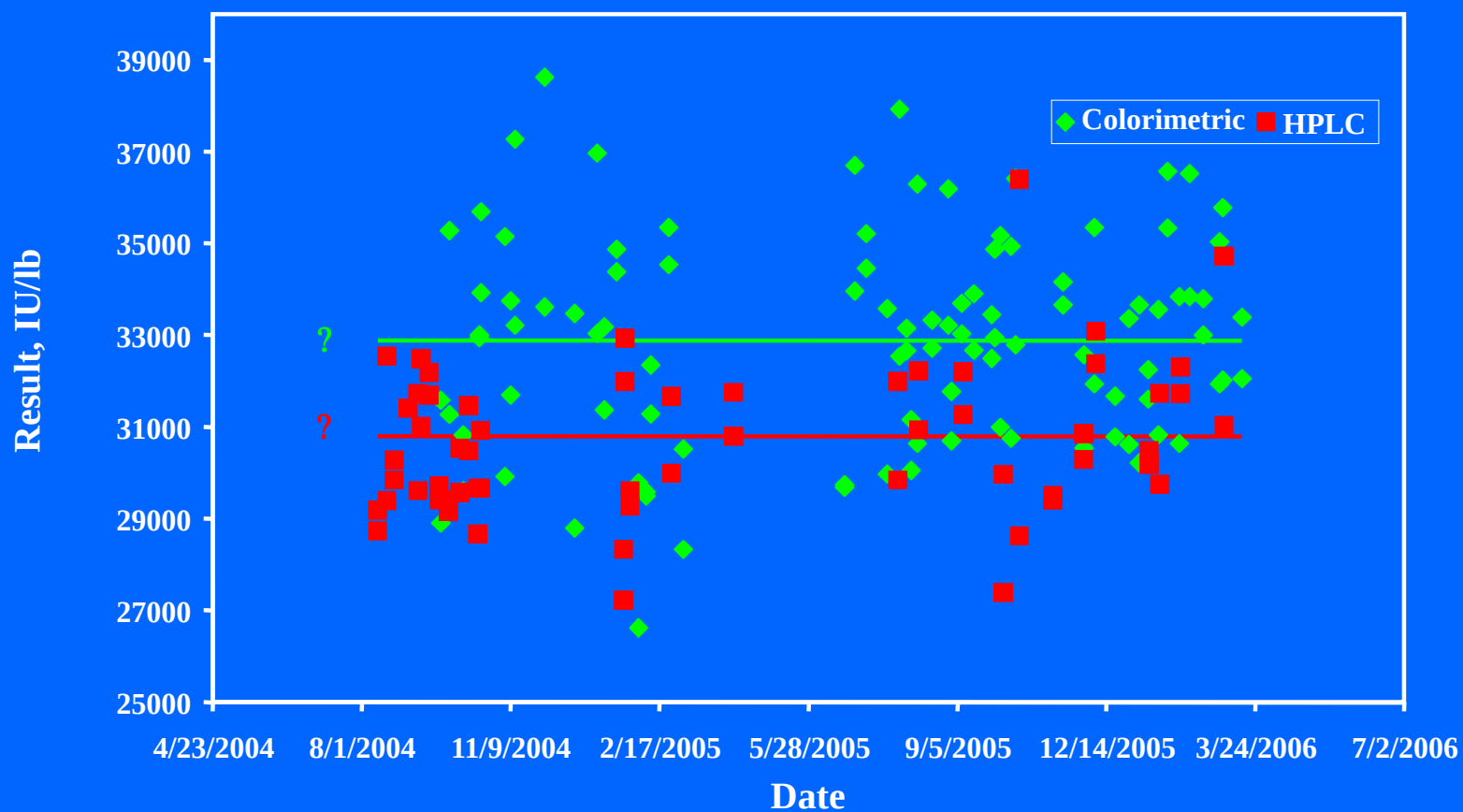
Vitamin Premixes





HPLC vs. Colorimetric Method

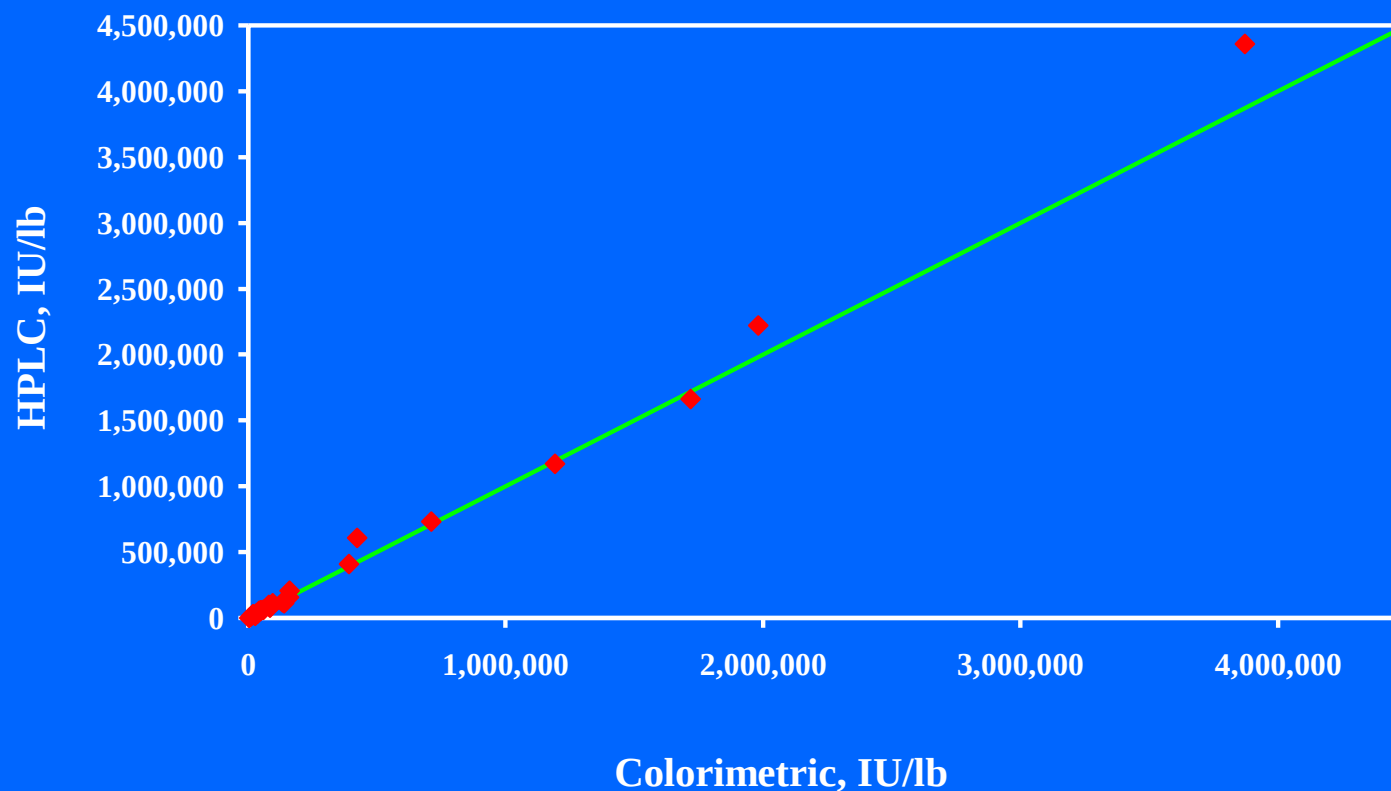
Colorimetric vs. HPLC, QC Sample





HPLC vs. Colorimetric Method

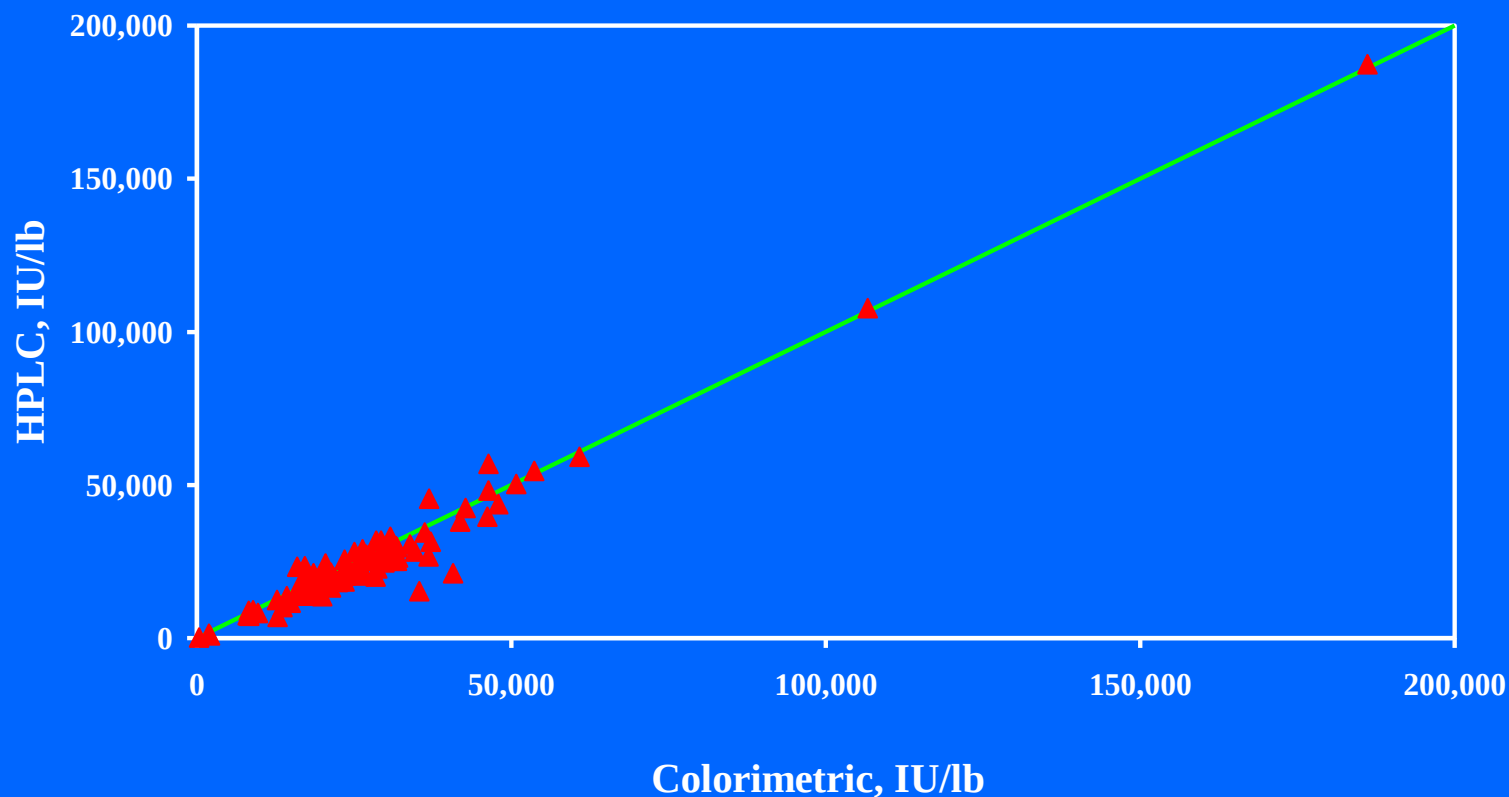
HPLC vs. Colorimetric, "Meal" Samples





HPLC vs. Colorimetric Method

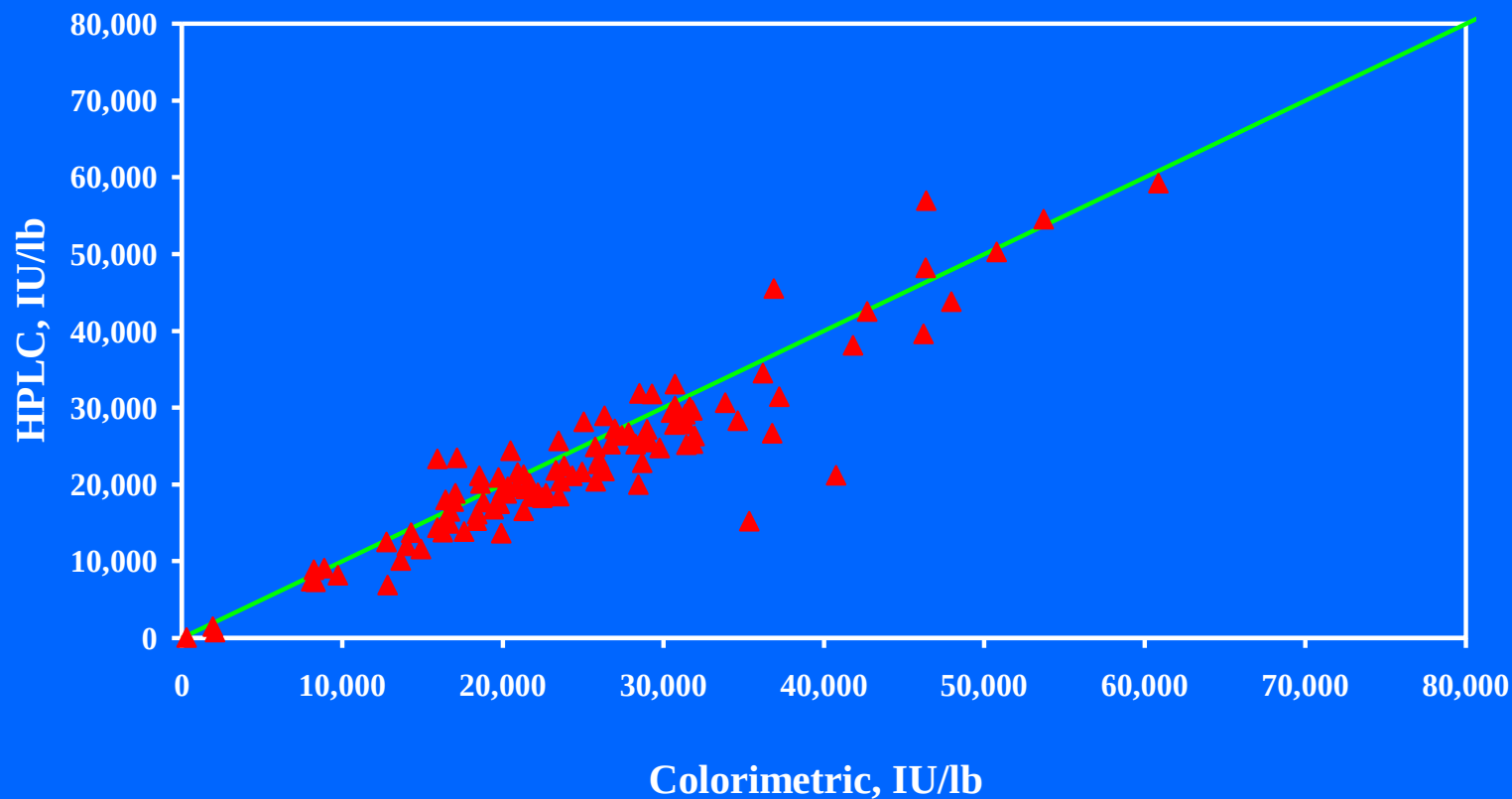
HPLC vs. Colorimetric, Pelleted Samples





HPLC vs. Colorimetric Method

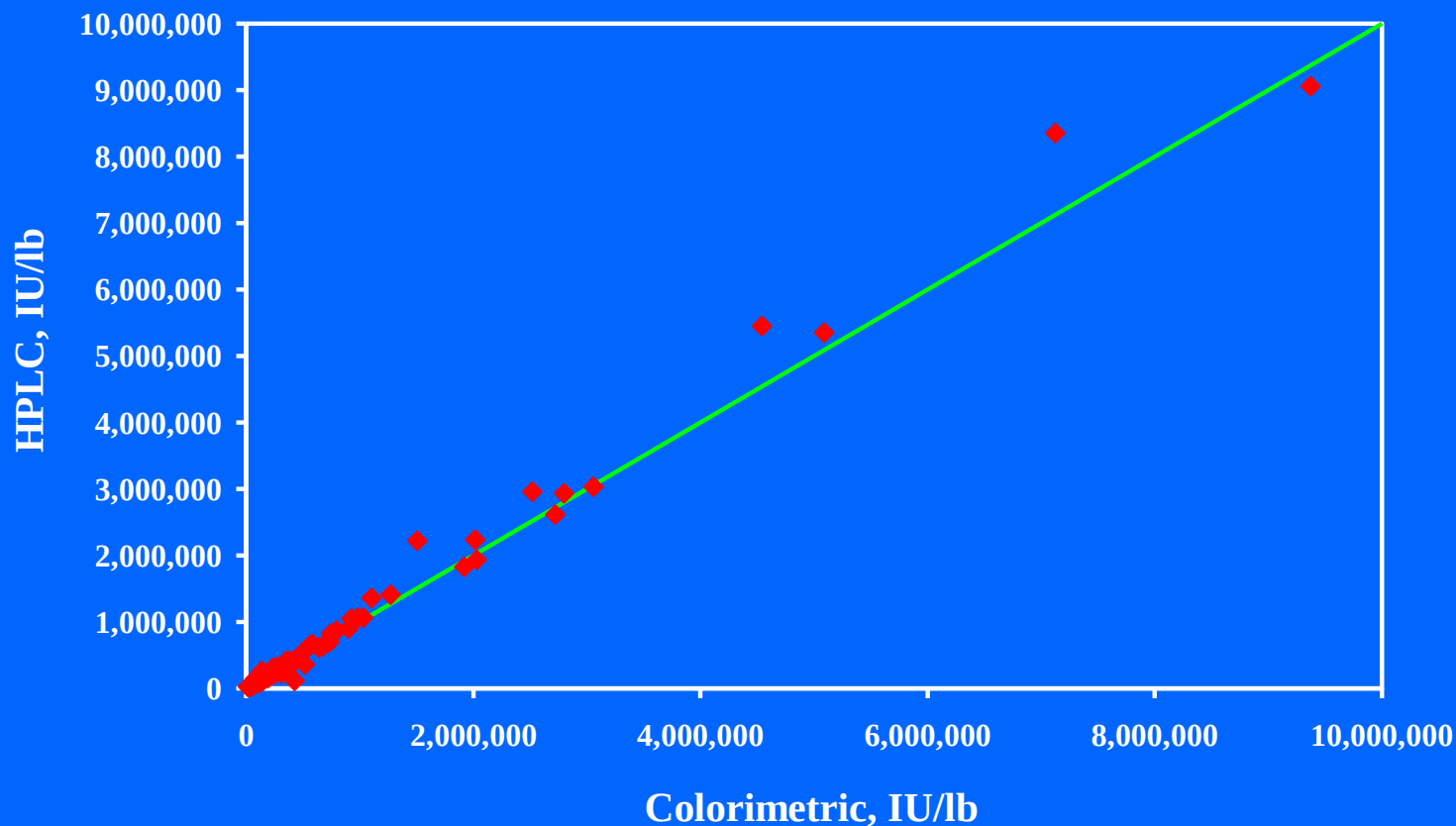
HPLC vs. Colorimetric, Pelleted Samples





HPLC vs. Colorimetric Method

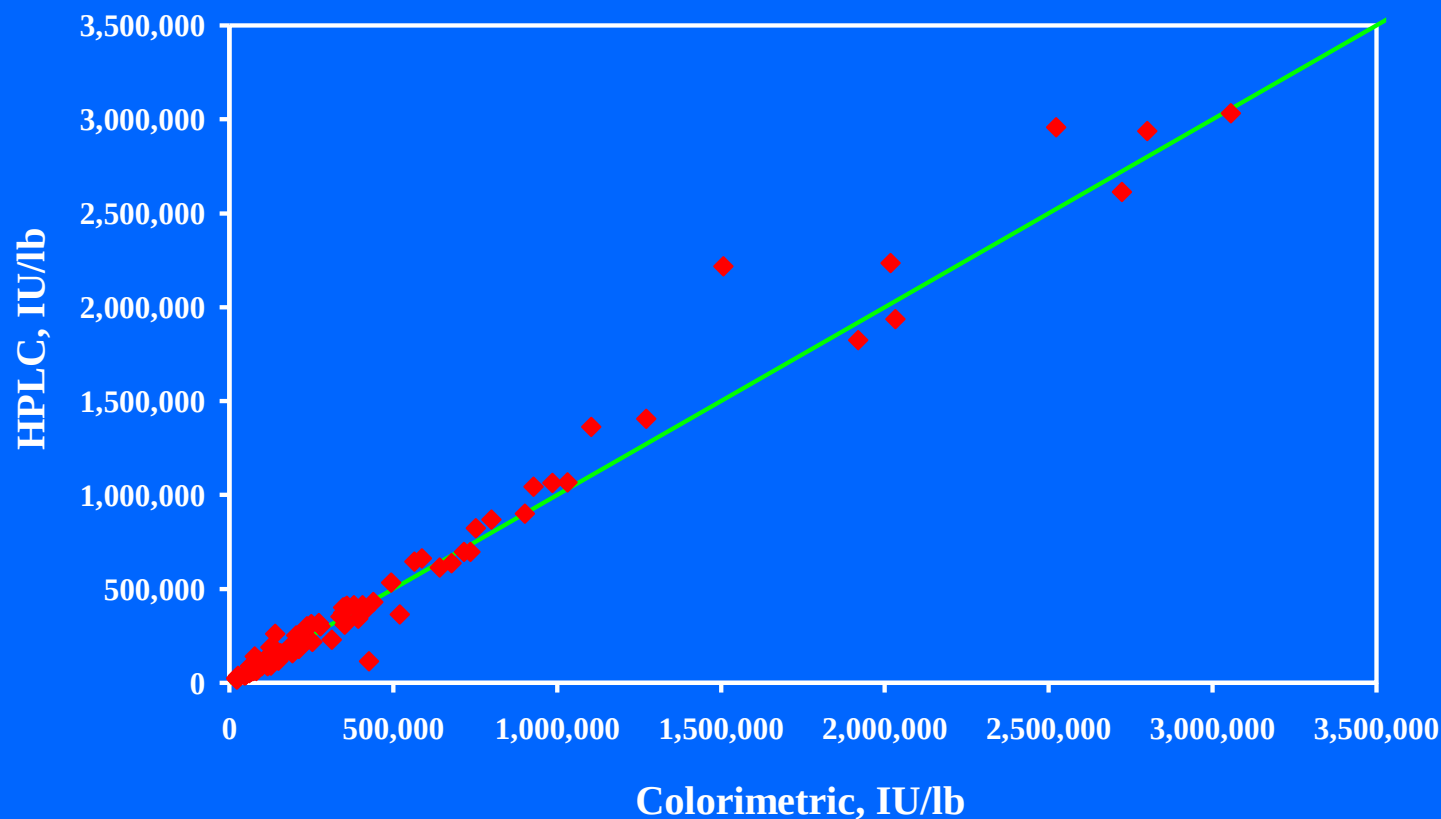
HPLC vs. Colorimetric, Mineral Samples

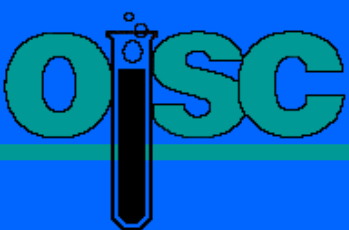




HPLC vs. Colorimetric Method

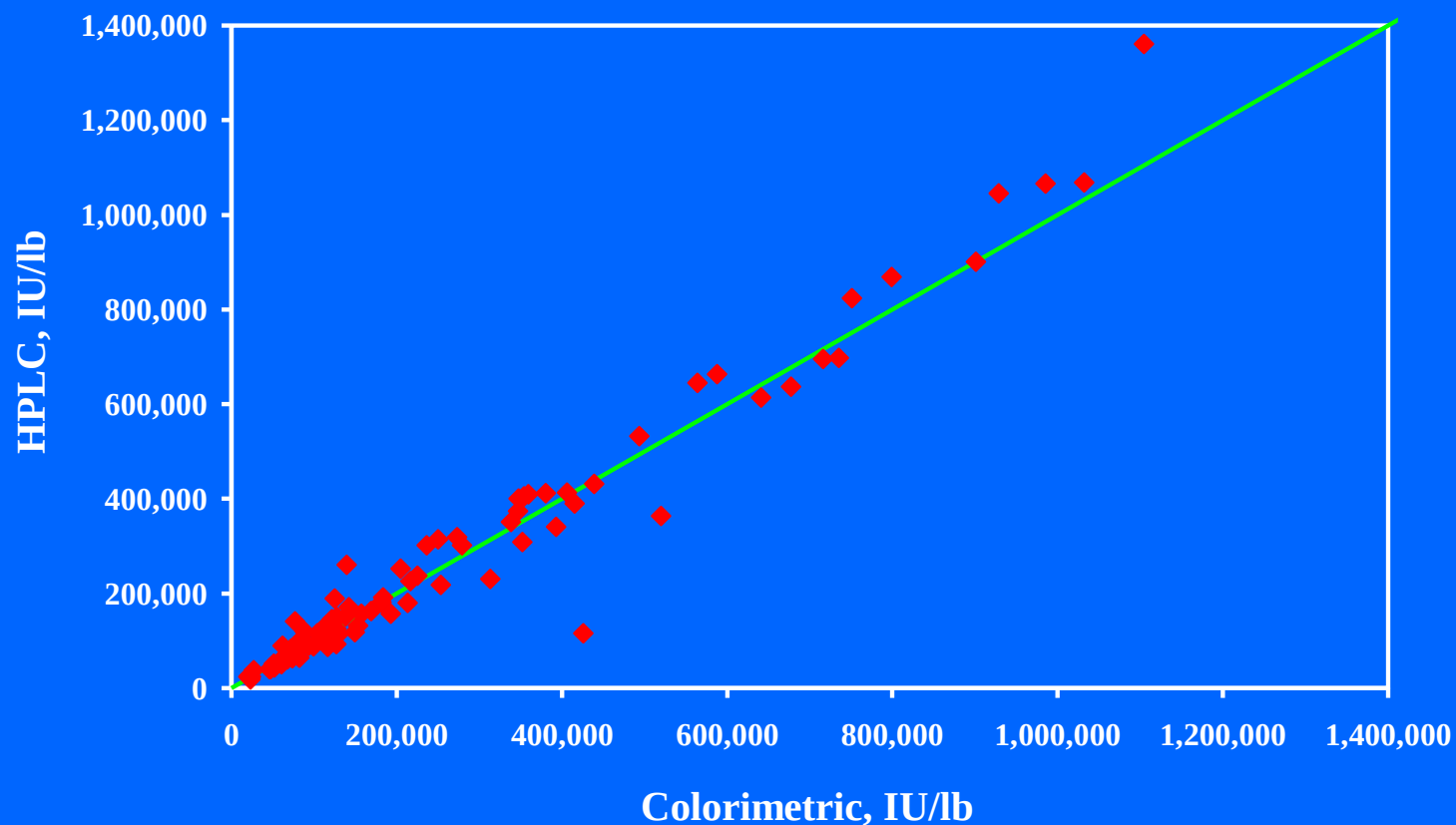
HPLC vs. Colorimetric, Mineral Samples





HPLC vs. Colorimetric Method

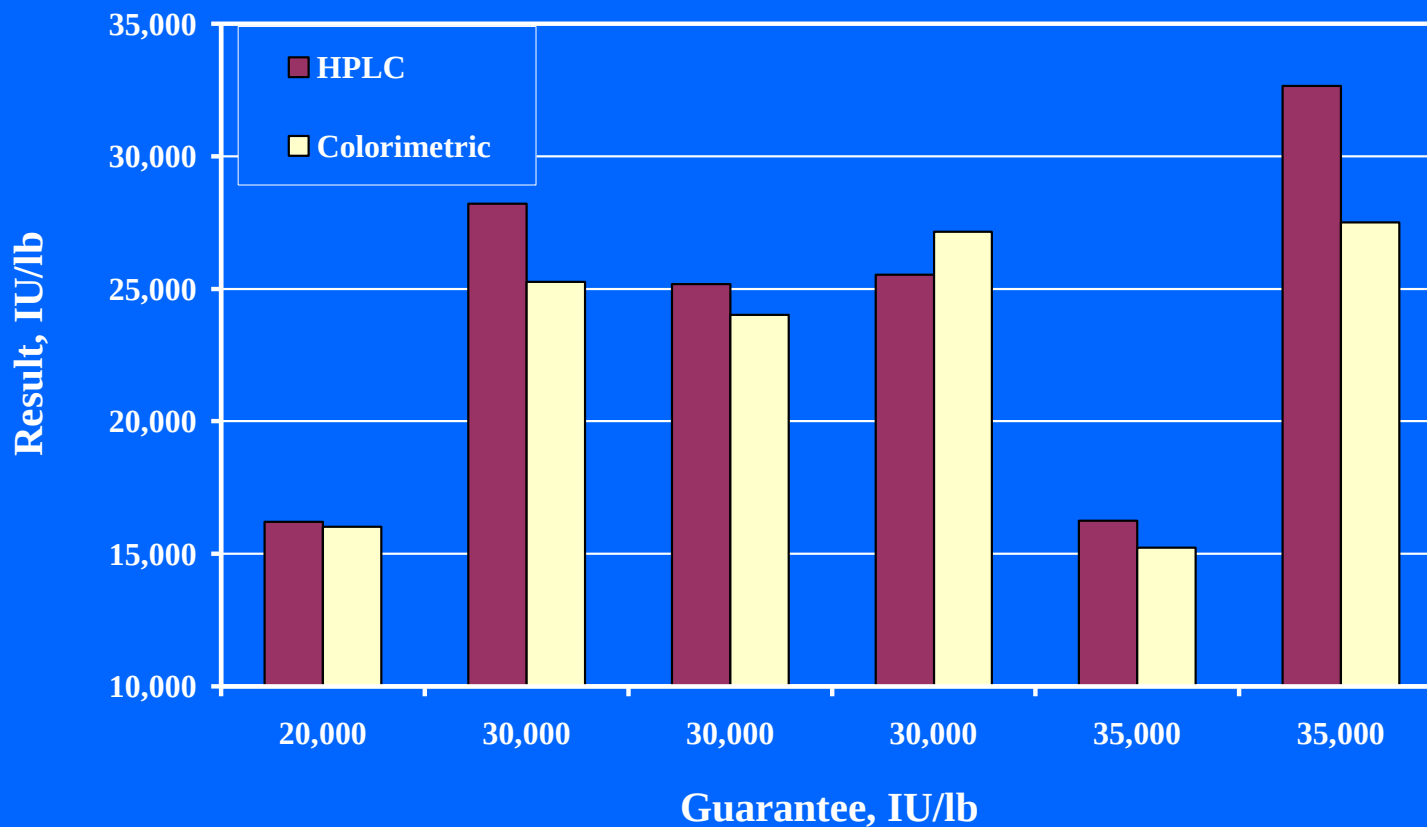
HPLC vs. Colorimetric, Mineral Samples

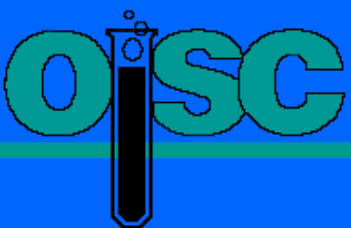




HPLC vs. Colorimetric Method

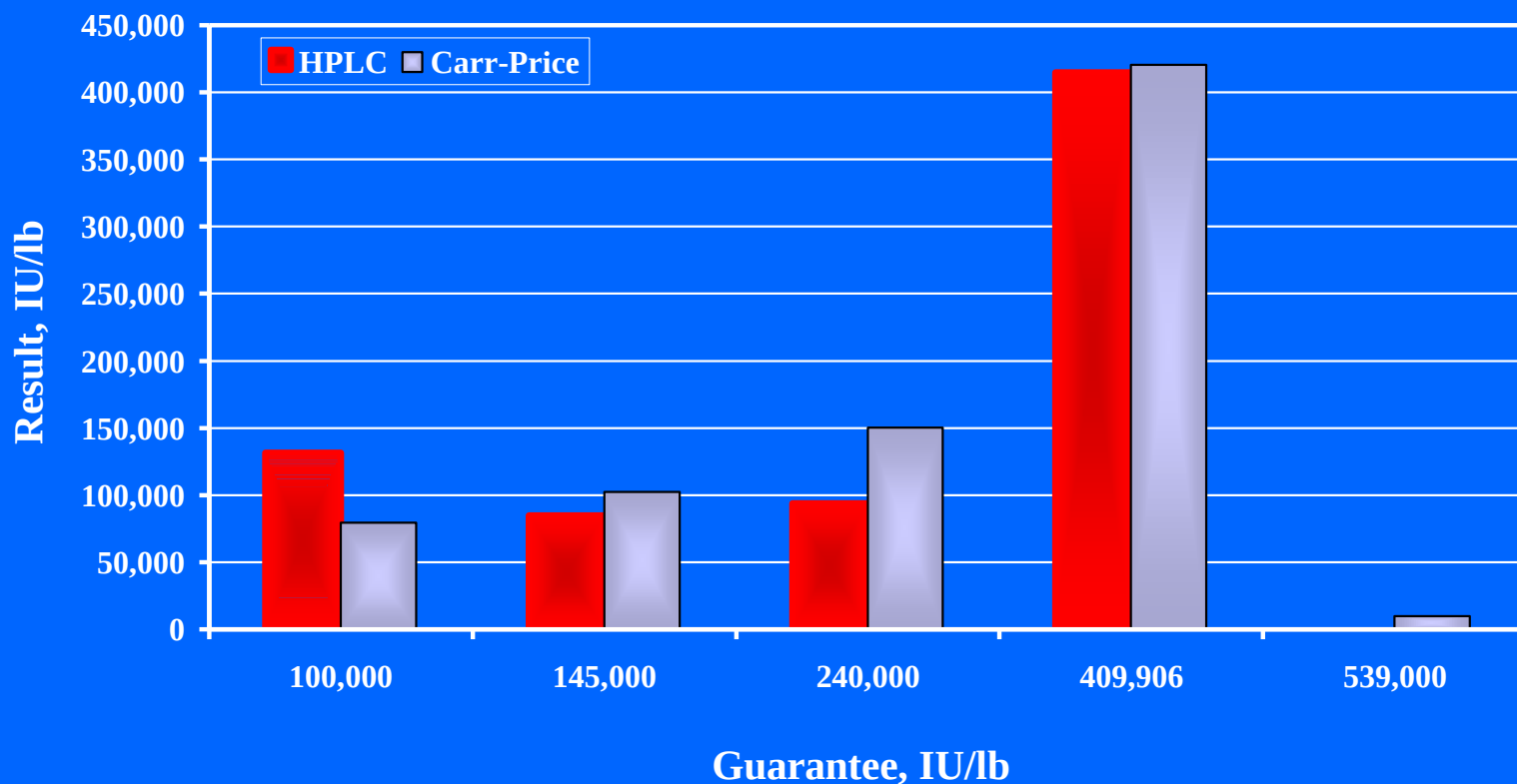
HPLC vs. Colorimetric, Dry Milk Replacers





HPLC vs. Colorimetric Method

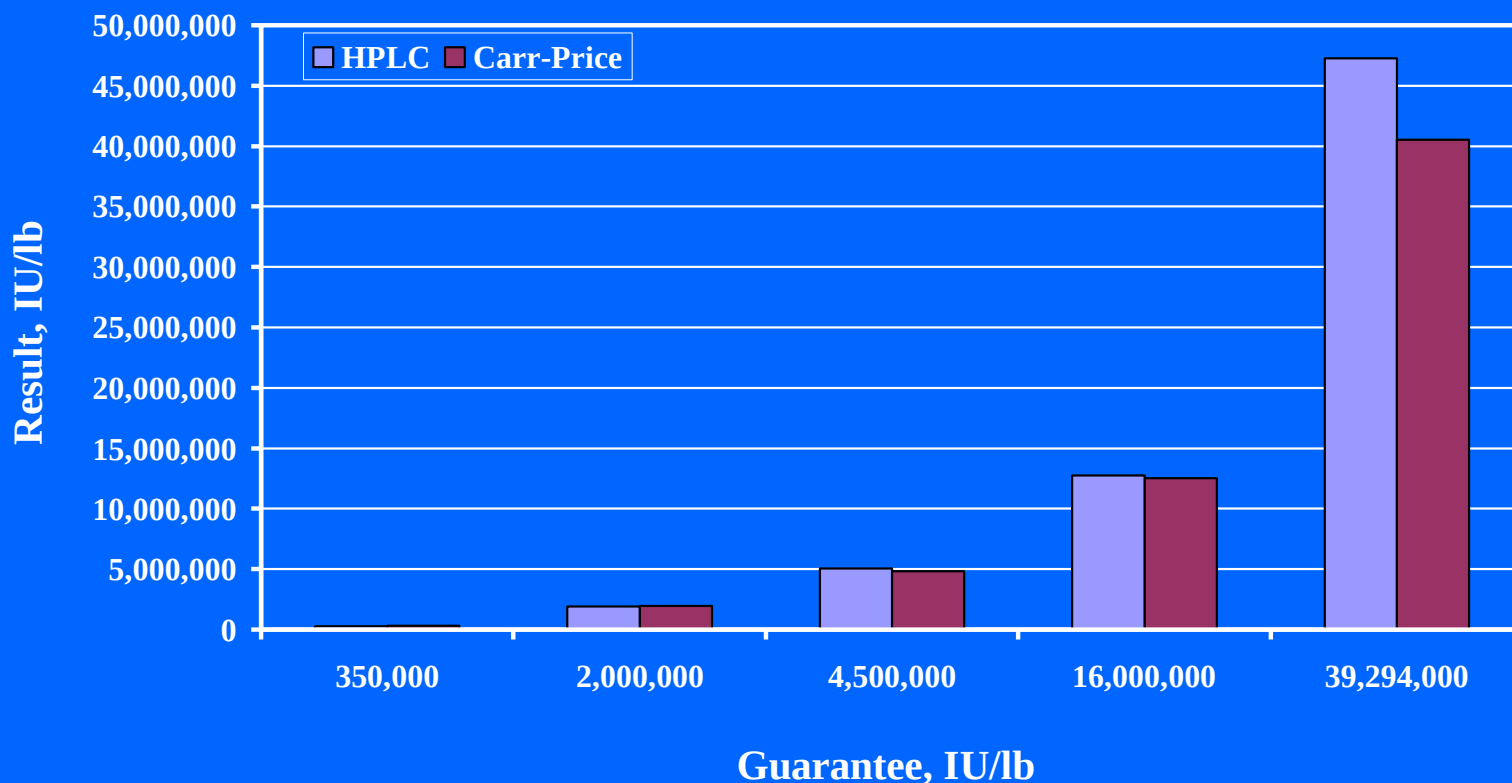
HPLC vs. Colorimetric, Liquid Samples





HPLC vs. Colorimetric Method

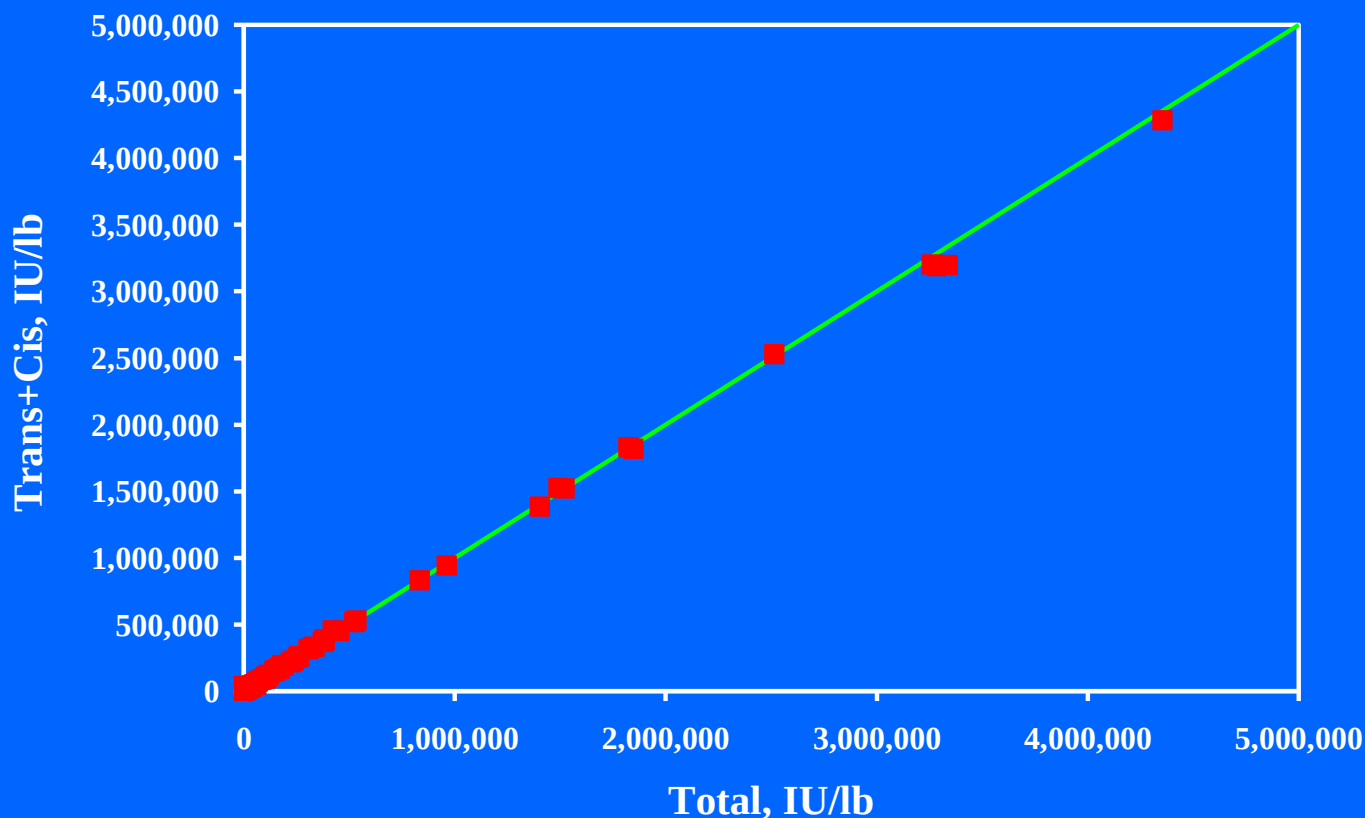
HPLC vs. Colorimetric, Vitamin Premixes





Cis- Isomer Separation

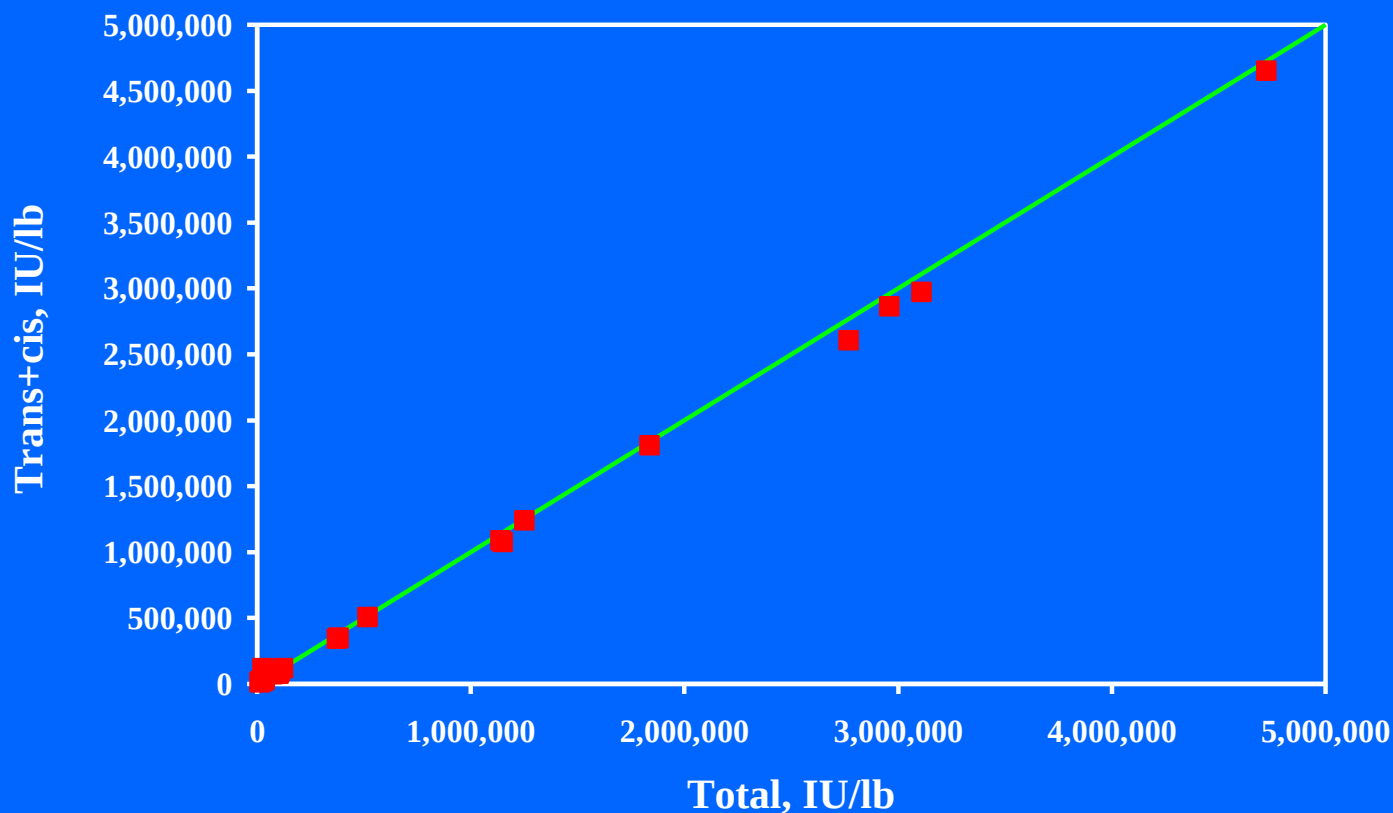
Quantitation w/ and w/o Cis Isomer Separation
Room Temperature Saponification





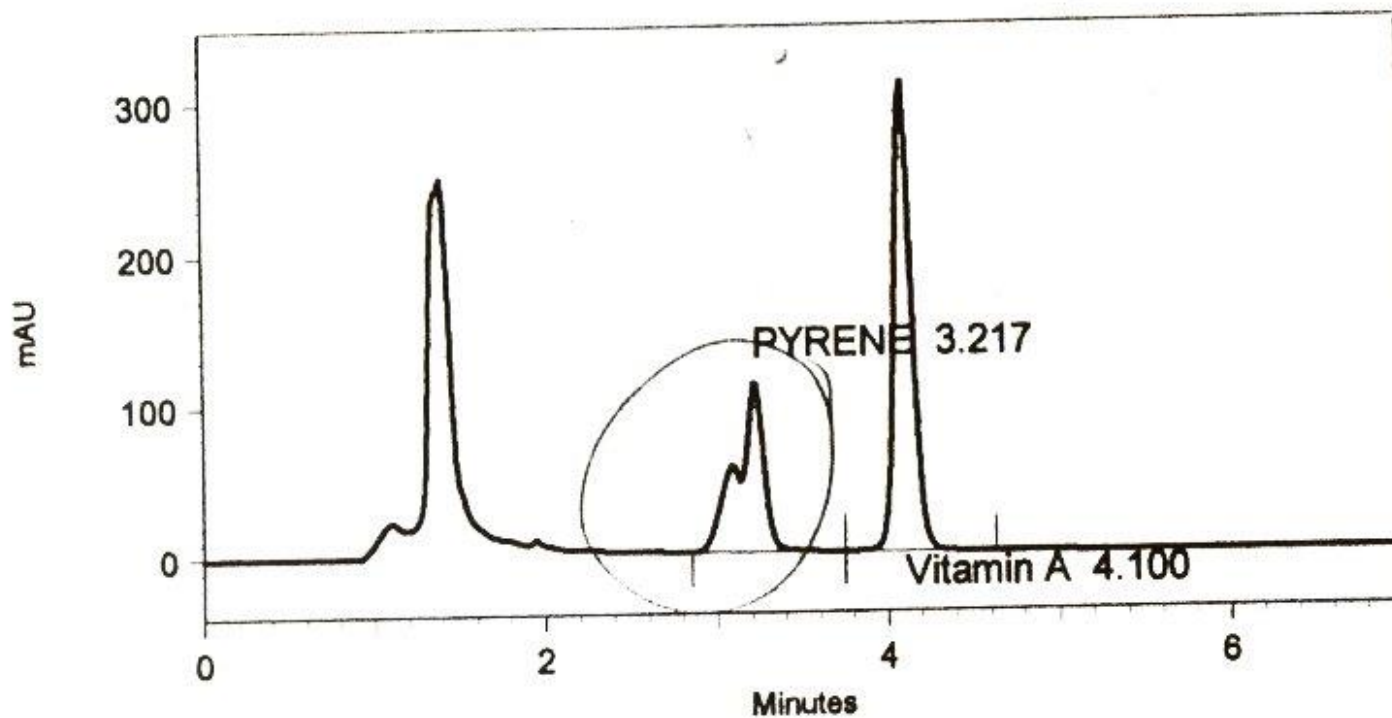
Cis- Isomer Separation

Quantitation w/ and w/o Cis Isomer Separation
Hot Saponification





Sample Matrix Interference





Chromatographic Conditions

Column – **Zobax SB-C18, 3.5 μm , 150mm x 4.6 mm**
 or Vydac 201TP54, 250 mm x 4.6 mm, 5 μm
 or any suitable reverse-phase column

Detector – **UV6000LP**

Wavelength – **326 nm**

Mobile phase – **95:5 Methanol:Water**

Flow Rate – **1.0 mL/min.**

Injection – **10 μL**

Summary

- Good agreement with colorimetric method for most samples and matrices
- Hazardous reagents eliminated
- High sample throughput (~30/run)
- Method currently used to screen samples
- Approximate method range from 5,000 to 114,000,000 IU/lb

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OSC Acknowledgement

- Beth Anne Woody



Colorimetric vs. HPLC, QC Sample

