

International Vitamin Conference
May 15, 2014- Washington DC

Pharmacopeial Perspectives on Vitamin Analysis

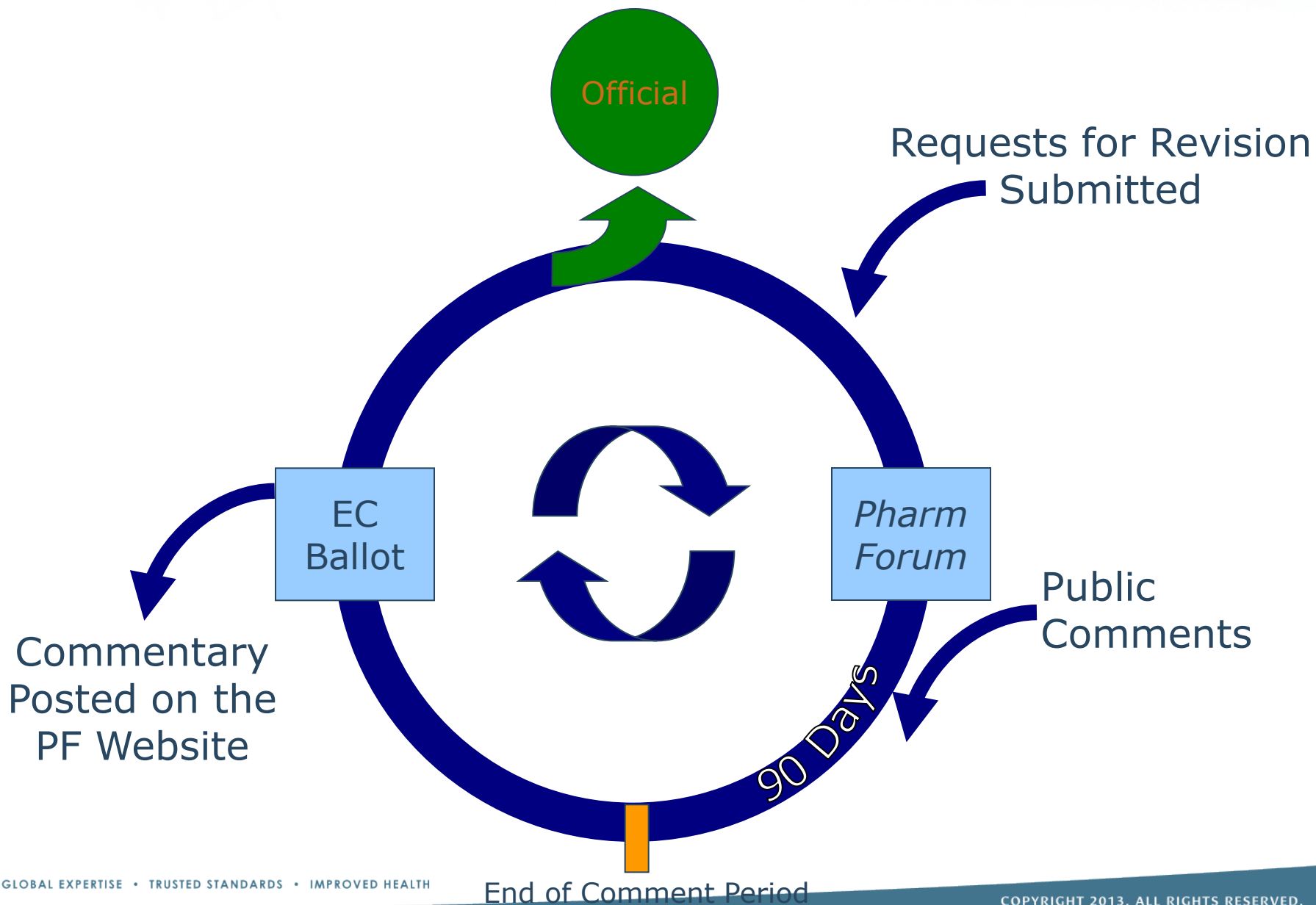
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Global Science and Standards Division
USP

Revision Process



www.USPPF.com

USP Standards Setting Process



USP-NF Revision Process



USP Dietary Supplement Monographs

Test \ DS Type	Vitamin	Minerals	Non Botanicals	Botanicals
Identity	IR, HPLC RT, UV, Chemical	Chemical	IR, HPLC RT, UV, Chemical	Microscopy, TLC, HPLC, GC
Purity/ Contaminants	Chromatographic purity, Limit Tests, Microbial, Heavy Metals	Chemical Limit tests, Limit of foreign metals by AA, ICP	Chrom. purity, Limit Tests, Microbial, Heavy Metals, PCBs-Dioxins	Toxins, Aflatoxins, Heavy Metals, Pesticides, Foreign Matter, Residue On Ignition, Microbial, Negative Markers
Performance	Packaging, Labeling, Uniformity, Dissolution, Disintegration	Packaging, Labeling, Uniformity, Dissolution, Disintegration	Packaging, Labeling, Uniformity, Dissolution, Disintegration	Packaging, Labeling, Extractable Matter, Uniformity, Dissolution, Disintegration
Strength/ Composition	Spectroscopy, HPLC, Microbial	AA, ICP, Titration	Spectroscopy, HPLC, Titration	HPLC, GC

Modernization Prioritization Scheme

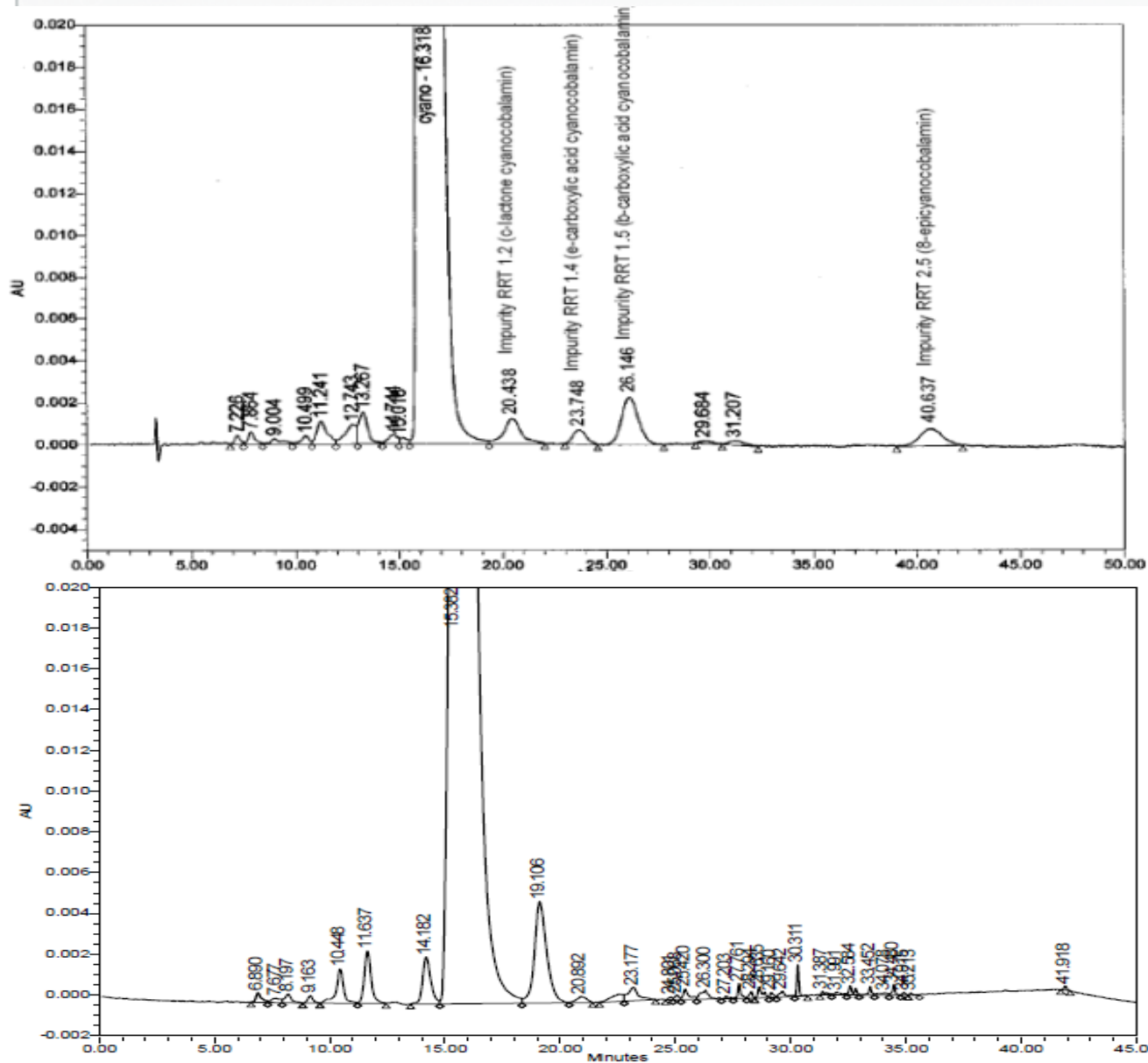
- ▶ Monograph Needs
 - Replace
 - Non-Specific Assay
 - Titrations
 - UV-Vis
 - Wet Chemistry
 - Microbial Assay
 - Non-Value Added Procedures
 - Melting Point
 - pH
 - Outdated Technology
 - TLC Impurity procedures
 - Packed-Column GC
 - Hazardous Tests
 - Toxic Solvents
 - Odor Tests
 - Add
 - Missing Impurity Procedures
 - Second Identification Procedures
 - Update
 - Older Chromatographic Reagents
 - Use Common Assay and Impurity Procedures
 - Include Multidimensional Detectors
- ▶ Regulatory Needs
 - FDA Priorities
- ▶ Public Needs
 - Public Exposure
 - Unit Sales
 - Drug Products Sales
 - Public Danger
 - FDA Requests
 - Published Health Threats
- ▶ Industry Needs
 - Reference Standard Sales
- ▶ USP Needs
 - Cost Containment
 - Family of Monographs
 - Full Monograph (not just parts)

- Necessary to maintain relevance
- Keep up with new technologies

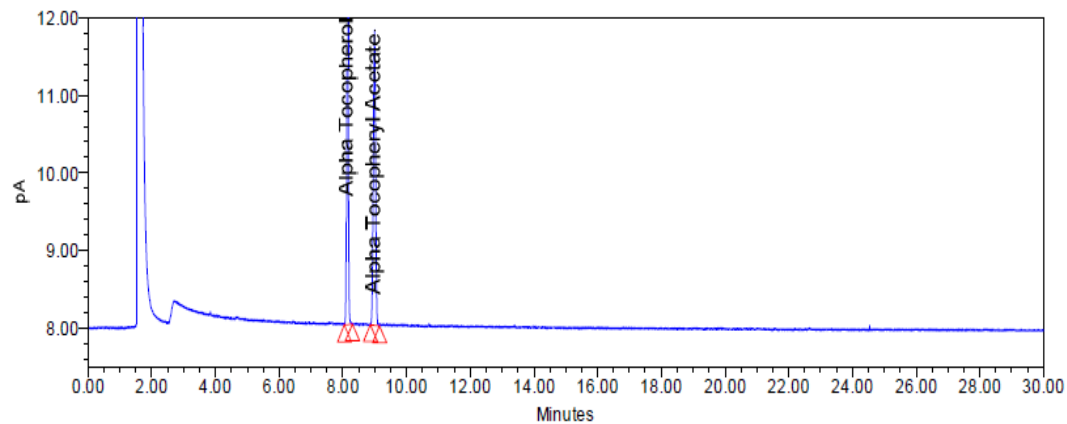
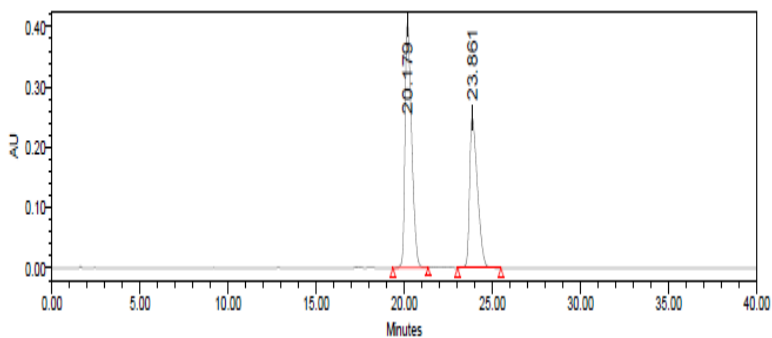
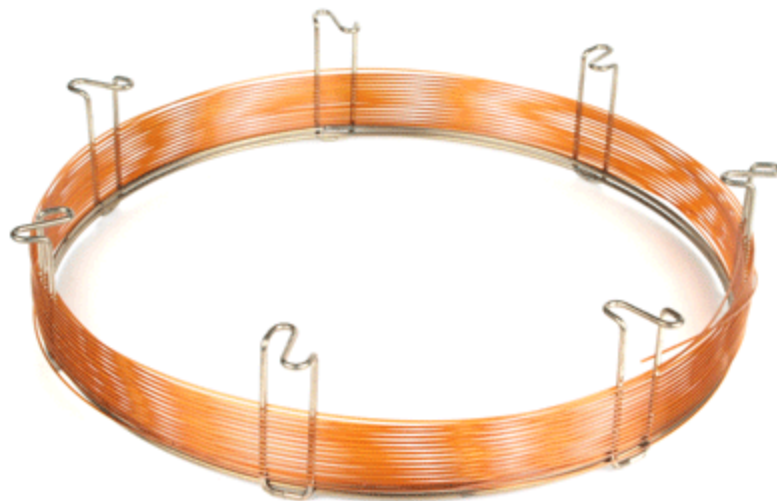
USP-NF Modernization Revision Process



Cyanocobalamin and Hydroxocobalamin



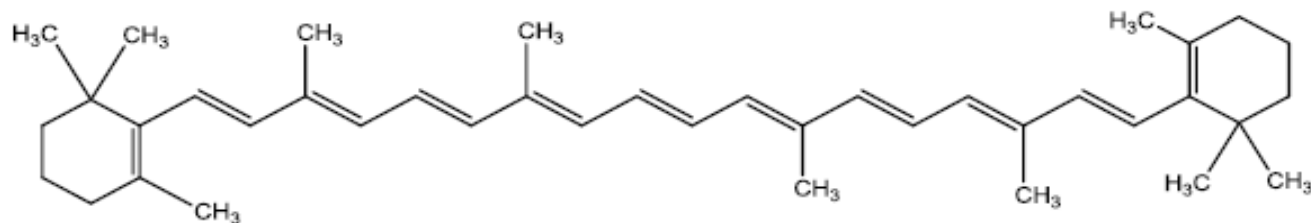
Vitamin E: GC packed column to capillary



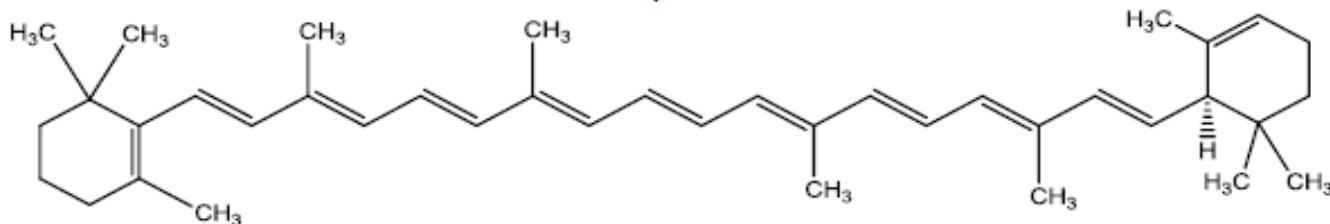
SampleName: Sus_Suit_Sol_Sample_ID: 15112016-00E_Vial B-2; Injection: 1; Channel: Description: Data

Modernizations: Beta carotene

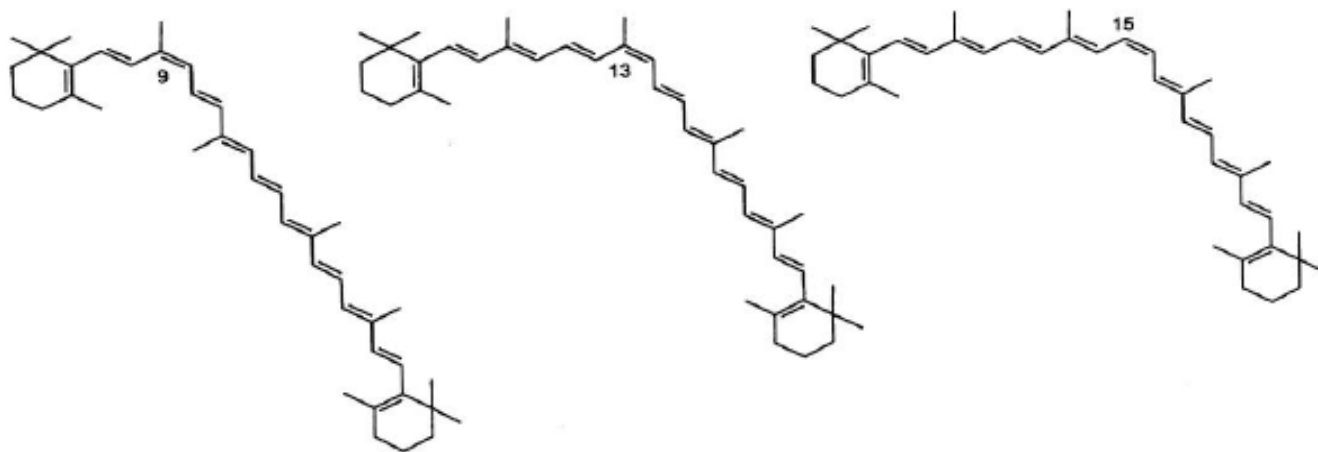
all trans-Beta Carotene



all trans-Alpha Carotene



Various *cis*-isomers

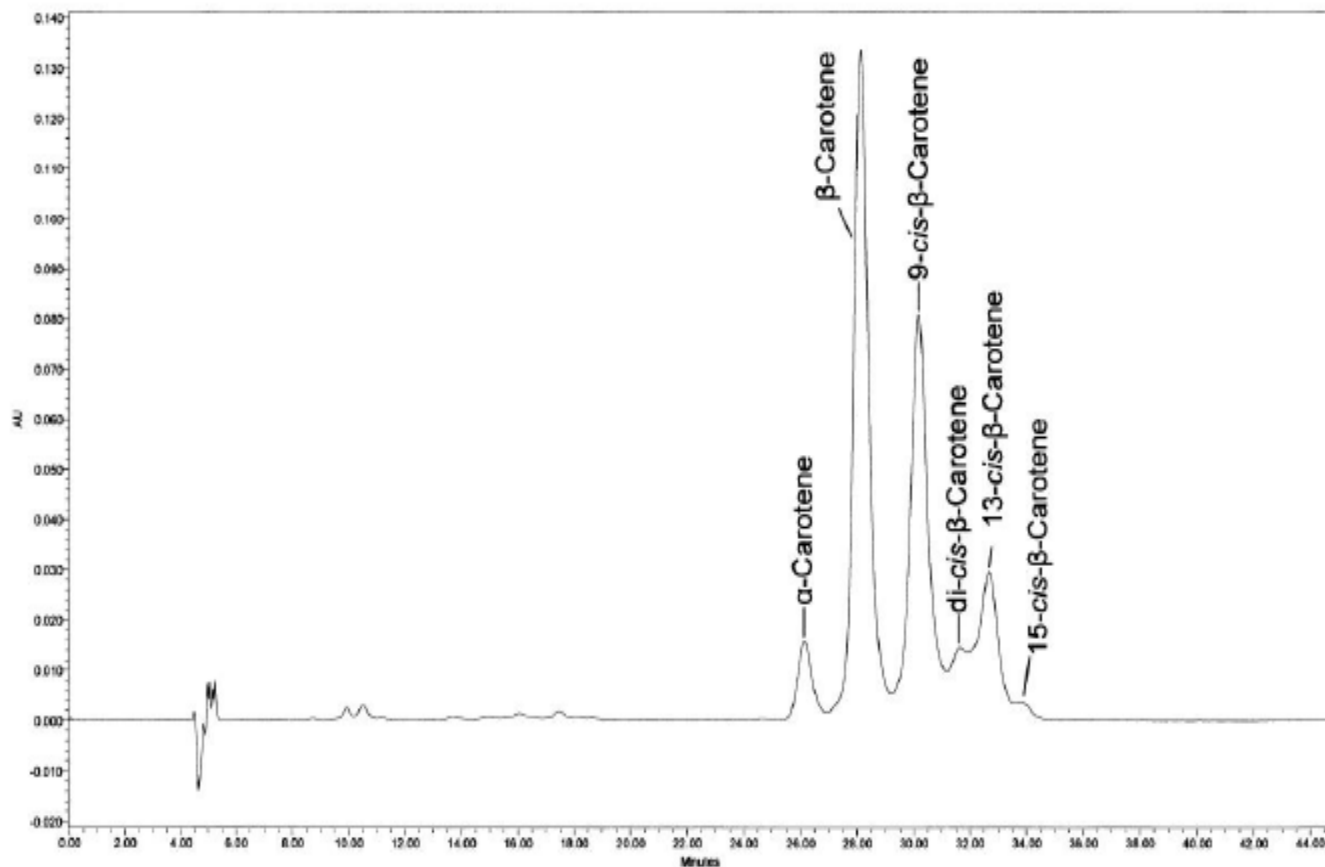


Modernizations: Beta Carotene

- ▶ Most of the Beta Carotene supplements are labeled in terms of Vitamin A units
- ▶ Provitamin A conversion in supplements is based on *all-trans*
- ▶ Conversion to Vitamin A is different for the *all-trans* vs *cis* forms
- ▶ *Cis* forms seem to gain interest as antioxidant and other actions
- ▶ Trans is predominant in fruits, vegetables, fermentation and synthetic
- ▶ Cis is significant in some algae (*Dunaliella*) and beta carotene preparations (beadlets)
- ▶ Spectrophotometric assay with Iodine catalyzer did not provide specific trans/cis content
- ▶ Therefore there was a need for specific assay

Modernizations: Beta Carotene

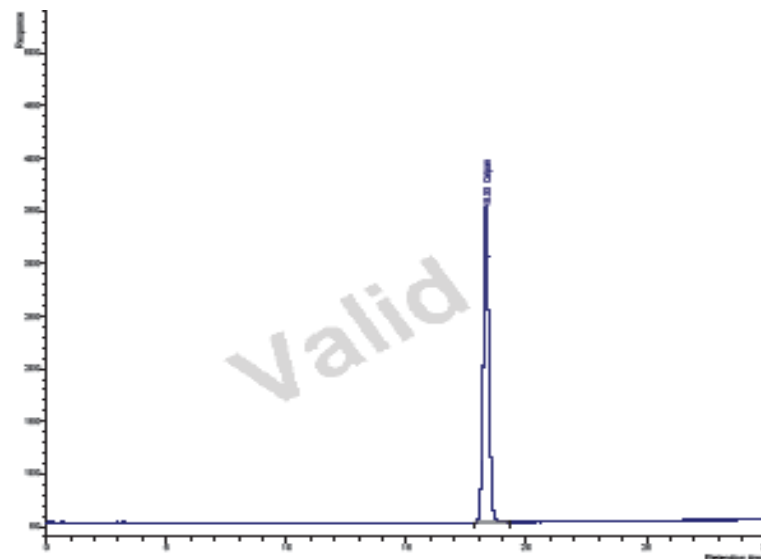
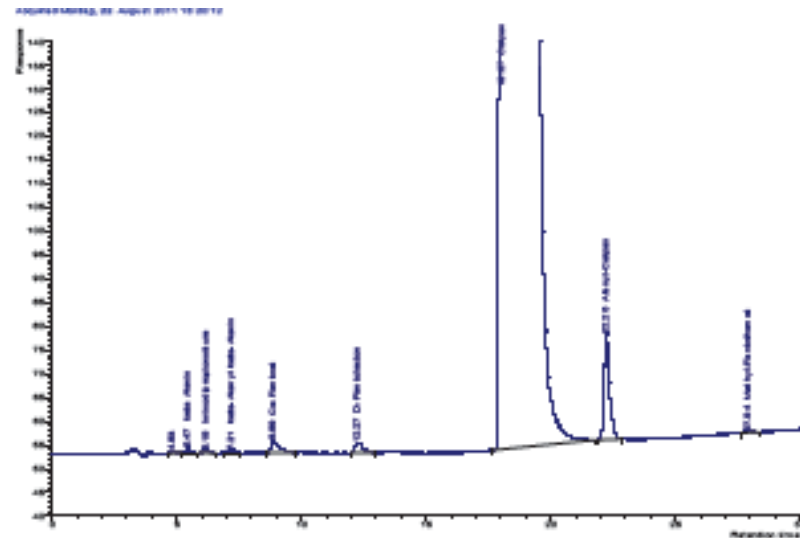
The typical chromatogram for USP Beta Carotene System Suitability RS presented in RSCEP 5488:



Calcium Panthotenate



Nitrogen determination
by Kjeldhal to HPLC for
Assay and Impurities



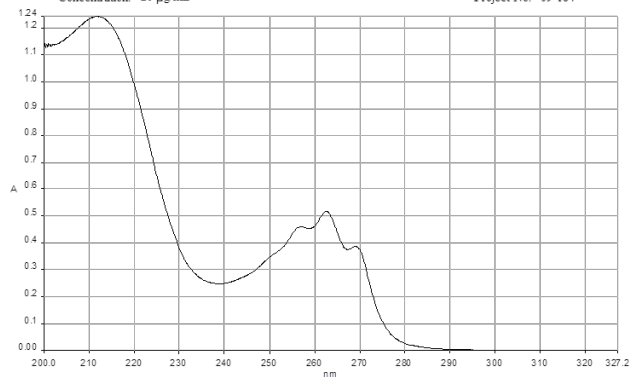
Niacin : UV + TLC to HPLC for assay and impurities

Project #: 09-184

Name: Niacin

USP Bulk Lot: B080288
Concentration: 20 µg/mL

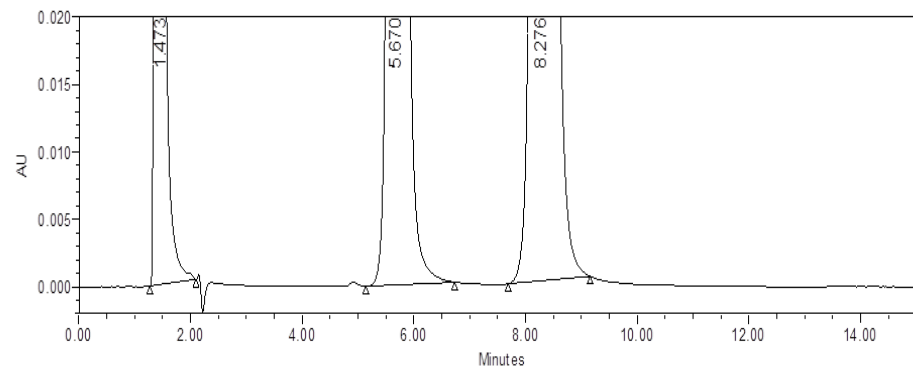
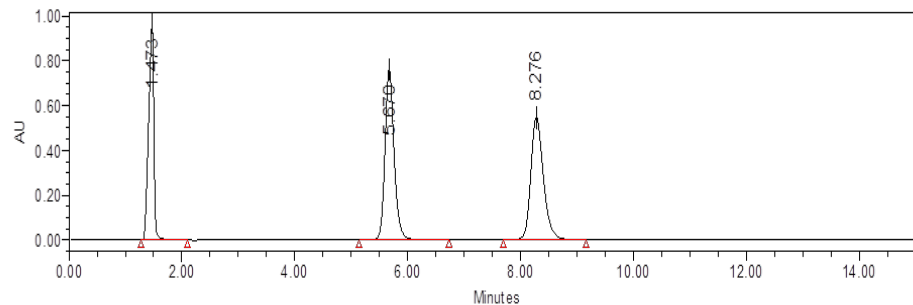
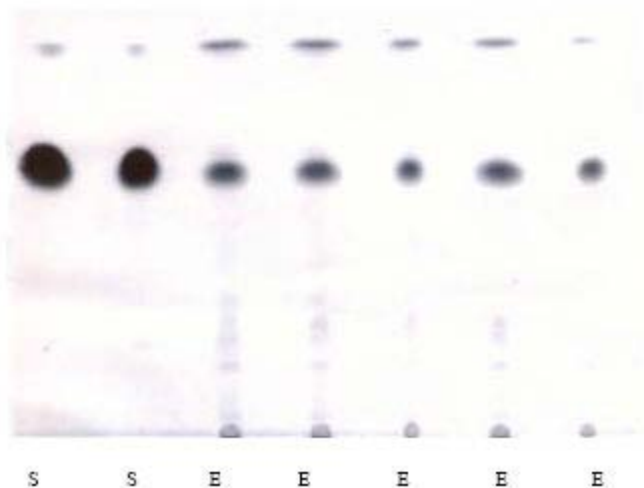
USP Lot: J
Project No: 09-184



RSL_WKBK-02
UV Figs

RSE Report

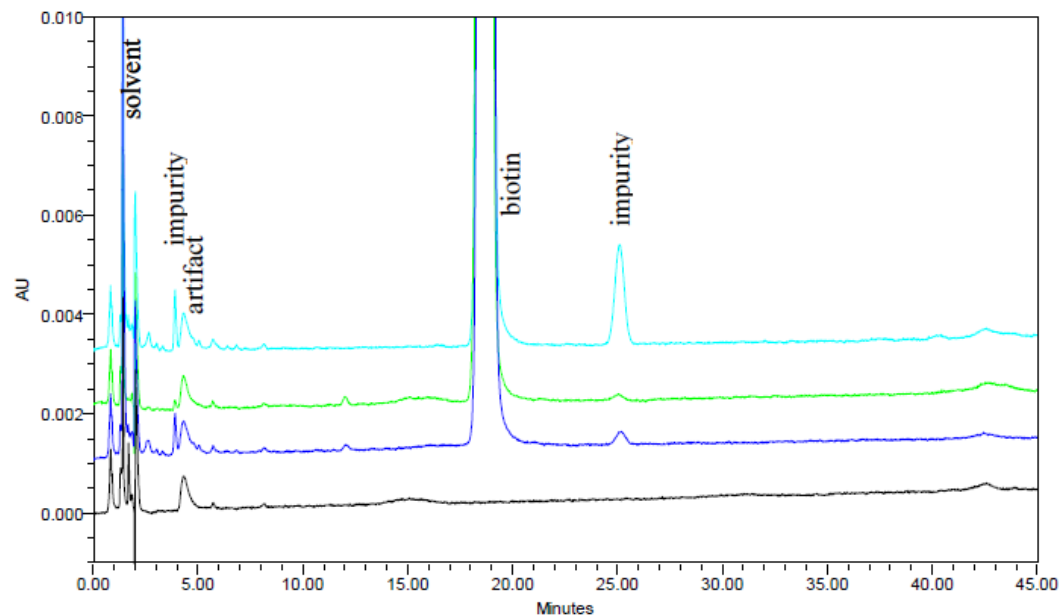
Page 10 of 11
Effective May 30, 2008



Peak Results

	Name	RT	RRT	Area	% Area	Height (µV)	USP Resolution	USP Plate Count	Int Type
1	pyridine	1.473	0.178	6180704	26.6896	970264		1076	Bb
2	6-hydroxynicotinic acid	5.670	0.685	8503337	36.7192	760297	17.72	6342	bb
3	niacin	8.276	1.000	8473719	36.5913	547687	7.45	6981	BB

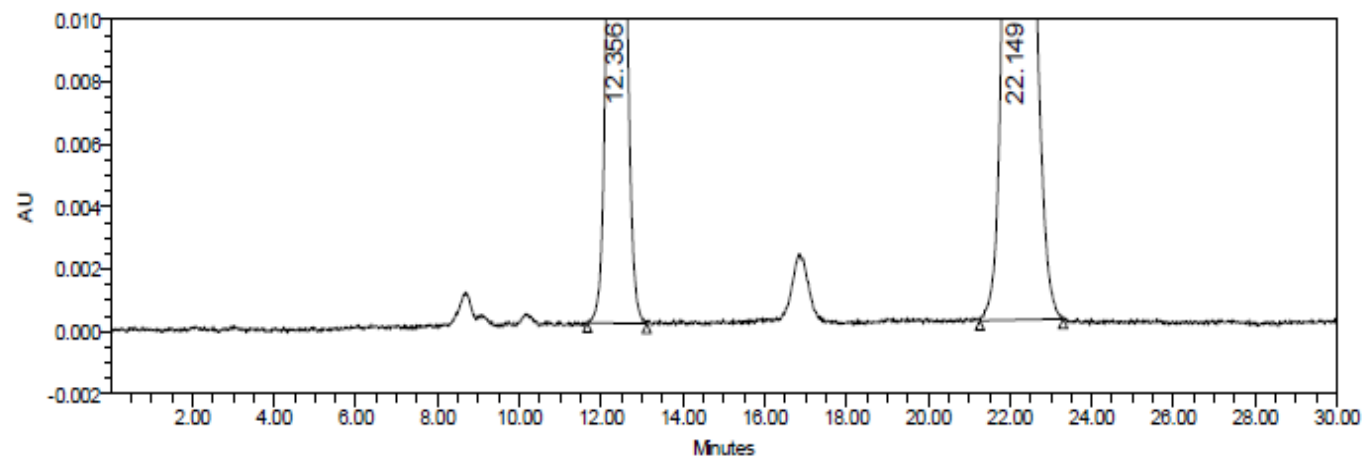
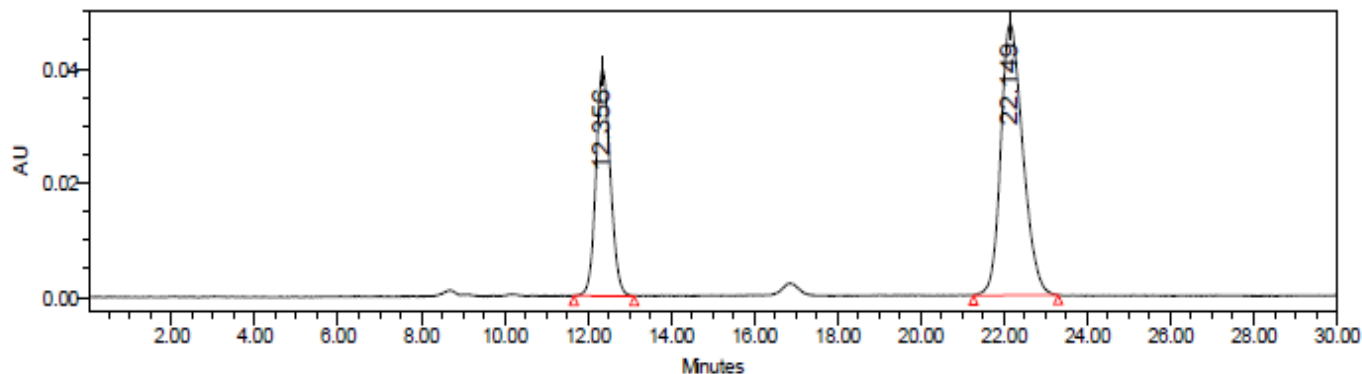
Biotin monograph: titration to HPLC



500 mg of sample

10 mg of sample

Vitamin A: introduction of Retinyl Palmitate RS



Peak Results

	Name	RT	Area	% Area	Height (μV)	USP Resolution	USP Plate Count	Int Type
1	retinyl palmitate	12.356	950348	35.1594	39830		6056	bb
2	retinyl acetate	22.149	1752623	64.8406	47488	11.97	8312	bb

1. <91> Calcium pantothenate Assay
2. <115> Dexpanthenol Assay
3. <171> Vitamin B12 Activity Assay
4. <411> Folic Assay
5. <441> Niacin or niacinamide Assay
6. <481> Riboflavin Assay
7. <531> Thiamine Assay
8. <551> Alpha Tocopherol Assay
9. <571> Vitamin A Assay
10. <581> Vitamin D Assay

Goals of Chapter Modernization

- ▶ Eliminate outdated procedures and/or impractical/time consuming techniques.
- ▶ Consolidate all the common analytical methods that are shared by many monographs into one place.
- ▶ Apply the redesigned format to the entire chapter where applicable.

<571> Vitamin A Assay

- ▶ Delete the test for articles containing tocopherol in the Chemical Method and replace it with a chromatographic method.
- ▶ Apply the redesigned style format for the entire chapter where applicable.
- ▶ Consolidate all the existing chromatographic procedures in the various monographs by migrating them to the Chromatographic Methods section. As a result, general chapter 571 will serve as a center for cross-references for all vitamin A procedures described in the individual monographs.
- ▶ 3 sample preparations:
 - DMSO-isooctane, neutral,
 - Saponification, basic
 - Lecithin/methanol/sulfuric/isooctane, acid
- ▶ 4 chromatographic systems:
 - 3 normal phase (Si and NH₂)
 - 1 reverse phase

<581> Vitamin D Assay

- ▶ Delete the Chemical Method and the Biological Method that are outdated and are no longer being used.
- ▶ Delete the current Chromatographic Method that is no longer applicable to any existing monographs.
- ▶ Apply the re-designed format to the entire chapter where applicable.
- ▶ . Consolidate all the existing chromatographic procedures in the various monographs by migrating them to the Chromatographic Methods section.

As a result, general chapter <581> will serve as a center for cross-references for all vitamin D procedures described in the individual monographs.

<551> Alpha Tocopherol Assay

- ▶ Replace the current <551> title “Alpha Tocopherol Assay” with “Vitamin E Assay” to broaden the assay scope which would include the alpha tocopheryl acetate and alpha tocopheryl acid succinate forms of vitamin E.
- ▶ Delete the alpha tocopherol Assay Procedure that is outdated and no longer being referenced in monographs.
- ▶ Apply the redesigned format to the entire chapter where applicable.
- ▶ Consolidate all the existing chromatographic procedures in the various monographs

<551> will serve as a center for cross-references for all vitamin E procedures described in the individual monographs.

<441> Niacin or Niacinamide Assay

- ▶ Delete the Microbiological Method. It is outdated and no longer referenced by the monographs.
- ▶ Apply the redesigned format to the entire chapter where applicable.
- ▶ Consolidate all the existing chromatographic procedures in the various monographs

<441> will serve as a center for cross-references for all Niacin or Niacinamide procedures described in the individual monographs.

<411> Folic Acid Assay

- ▶ Replace the current, stand-alone folic acid assay procedure in the chapter with newer procedures for the determination of folic acid as an ingredient of pharmaceutical formulations containing other active constituents.
- ▶ Consolidate all the existing chromatographic procedures in the various monographs by migrating them to this chapter, which will serve as a center for cross-references for all folic acid assay procedures described in the individual monographs

- Diagnostic Quality Control tool to identify variations in the manufacturing process
- Confirm the robustness of manufacturing process
- Predict availability for absorption
- Challenges with softgel capsules



Rupture Test for Soft Shell Capsules

1. Schizochytrium Oil Capsules
2. Saw Palmetto Capsules
3. Crypthecodinium cohnii Oil Capsules
4. Cod Liver Oil Capsules
5. Fish Oil Containing Omega-3 Acids Capsules
6. Pygeum Capsules

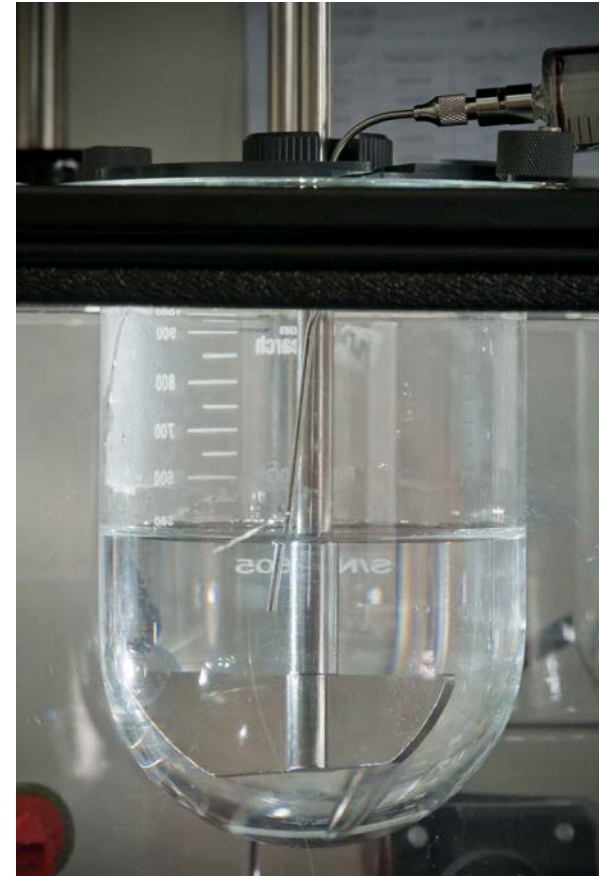
Rupture test for soft shell capsules

Medium: Water; 500 mL

Apparatus 2: 50 rpm.

Time: 15 min

- End point
 - The state of being broken, fractured, cracked.
 - There is no requirement for capsule's content release.



- All oil and water-soluble vitamins with minerals, prepared as tablets or capsules, are subject to the dissolution test and criteria described in the General Chapter <2040> for

- ✓ folic acid (if present Mandated for health claim)
- ✓ one index water-soluble vitamin
- ✓ vitamin A for oil soluble vitamins (PF 36(5) Sep-Oct 2010)

Davydova, N., Stipler E., Jin P., and Giancaspro G. *J. Pharm. Biomedical Anal.* 2010 Nov 2;53(3):295-301

[Online access]. DOI: 10.1016/j.jpba.2010.03.036.

- ✓ one index element

USP Apparatus 3

- Dissolution conditions for folic acid and index water-soluble vitamins (not suitable for minerals)

Test 2—

Medium: 45 mM citrate buffer, pH 6.0; 250 mL.

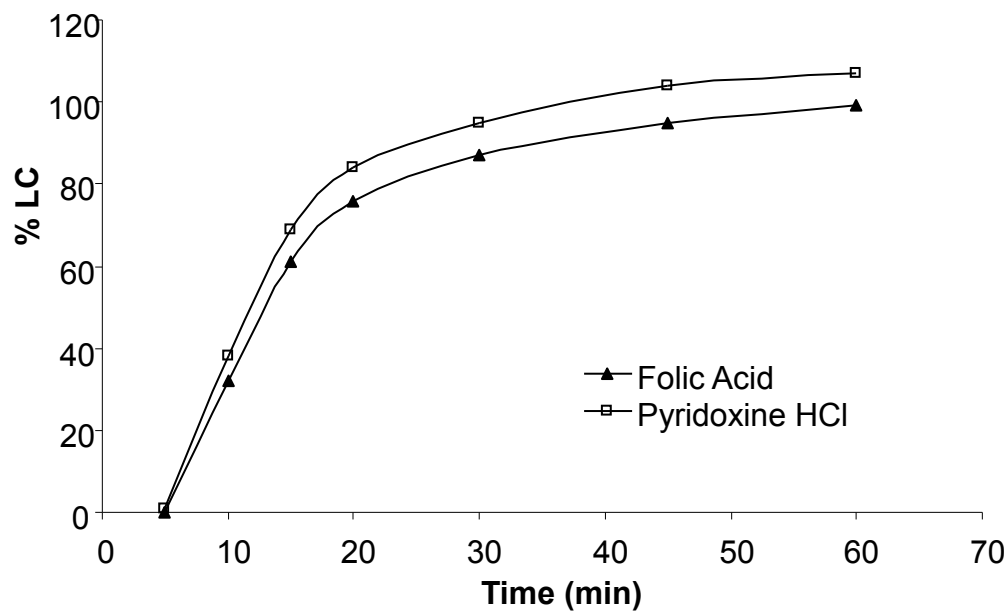
Apparatus 3: 30 dpm.

Screen (Top & Bottom): 56-mesh.

Time: 1 hour.

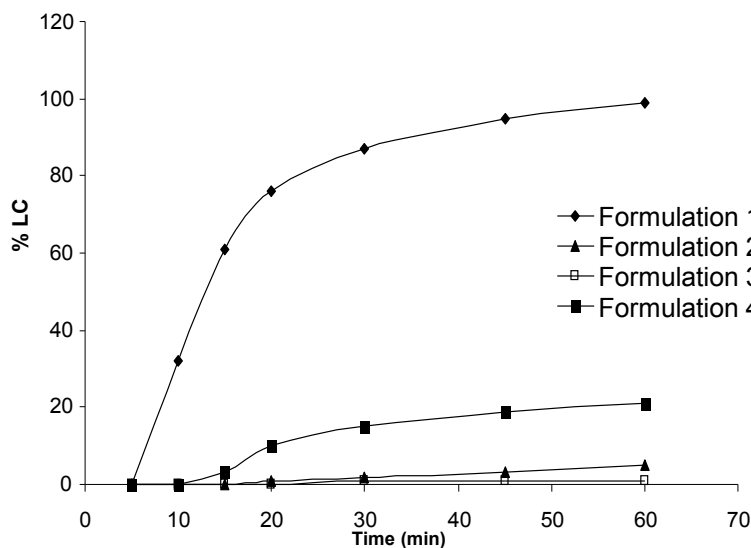
USP Apparatus 3

- The method works for some formulations

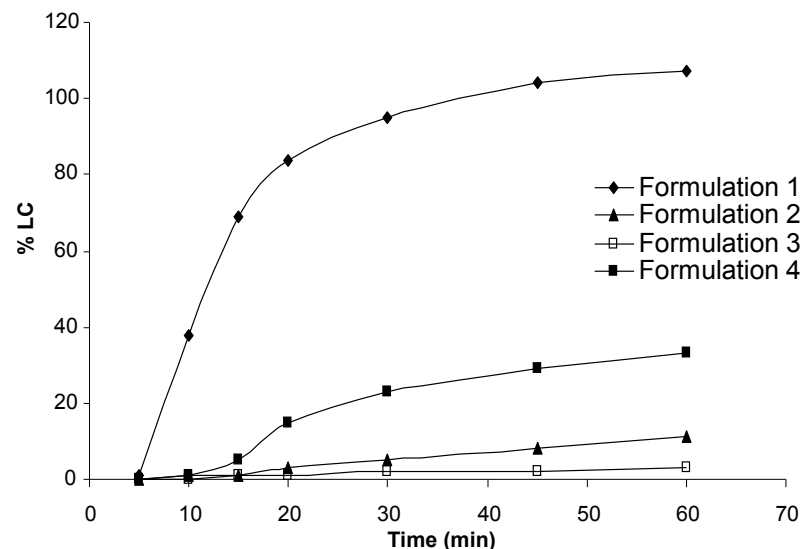


USP Apparatus 3

- The method has to be modified for evaluation of other soft gel capsules containing water- and oil-soluble vitamins with minerals



Folic Acid Release



Vitamin B6 Release

USP Apparatus 3 - Modifications

- Use of surfactants
 - ✓ Triton X -100: 0.25%; 0.5%; 1% in dissolution medium
- Use of enzymes
 - ✓ 1N HCl; SGF; SIF
- Mesh size
 - ✓ 56-mesh → 43-mesh
- Speed
 - ✓ 30 – 15 dpm

What's next?

- ▶ We want more UHPLC procedures!
- ▶ All in one?
- ▶ Yes, we wish, but practical and not too expensive to run

Questions

Thank You