



Ensuring a Method Is “Fit for Purpose”

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GMP Requirements

- cGMP requires that a person who manufactures a dietary supplement conduct at least one appropriate test or examination to verify the identity of each dietary ingredient that will be used in the manufacture of the dietary supplement.
- Quality means that the dietary supplement consistently meets the established specifications for identity, purity, strength, and composition, and limits on contaminants...
- §111.320:
 - (a) You must verify that the laboratory examination and testing methodologies are appropriate for their intended use.
 - (b) You must identify and use an appropriate scientifically valid method for each established specification for which testing or examination is required to determine whether the specification is met.



GMP Requirements

- “Scientifically Valid” appears 76 times in Dietary Supplement GMPs.
 - What does “scientifically valid” mean?
 - “we believe a scientifically valid method is one that is accurate, precise, and specific for its intended purpose. In other words, a scientifically valid test is one that consistently does what it is intended to do.” (GMP Final Rule, p. 401).
 - Sounds awful lot like method validation.
 - GMPs do not require you to use and officially validated method, such as AOAC or USP.



Definitions

- **Method:** The detailed set of instructions, from the preparation of the test sample to the reporting of the results, that must be followed exactly for the results to be accepted for the stated purpose.
 - Sometimes the term “method” is used interchangeably with “technique.”
 - e.g. HPLC, GC, AA, etc.
 - Sometimes the term “method” simply refers to instrumental conditions.



Definitions

■ Validation

- Simple definition: the systematic process of demonstrating the performance of a method of analysis.
- Answers the question: is the method fit for its intended purpose?



Method Validation

- Different Levels of Validation
 - Single-Laboratory Validated Method
 - Full Collaborative Study
 - At least 8 labs (submitting useable data)



Challenges in Validation

Supplement Facts

Serving Size 2 Caplet(s)

Servings Per Container 45

Amount Per Serving		% DV
Vitamin A (50% as beta-Carotene 50% as Acetate)	5000.00 IU	100%
Vitamin C (as Ascorbic Acid & Calcium Ascorbate)	300.00 mg	500%
Vitamin D (as Cholecalciferol)	200.00 IU	50%
Vitamin E (as Natural d-alpha Tocopheryl Acetate)	100.00 IU	333%
Vitamin K (Phytonadione)	80.00 mcg	100%
Thiamin (Vitamin B-1)(as Thiamin Mononitrate)	50.00 mg	3333%
Riboflavin (Vitamin B-2)	50.00 mg	2941%
Niacin (as Niacin and Niacinamide)	50.00 mg	250%
Vitamin B-6 (as Pyridoxine Hydrochloride)	50.00 mg	2500%
Folic Acid	400.00 mcg	100%
Vitamin B12 (as Cyanocobalamin)	50.00 mcg	833%
Biotin	300.00 mcg	100%
Pantothenic Acid (as Calcium d-Pantothenate)	50.00 mg	500%
Calcium (as Calcium Carbonate and Calcium Citrate Malate)	200.00 mg	20%
Iodine (as Potassium Iodide)	150.00 mcg	100%
Magnesium (as Magnesium Oxide)	100.00 mg	25%
Zinc (as Zinc Oxide)	25.00 mg	167%

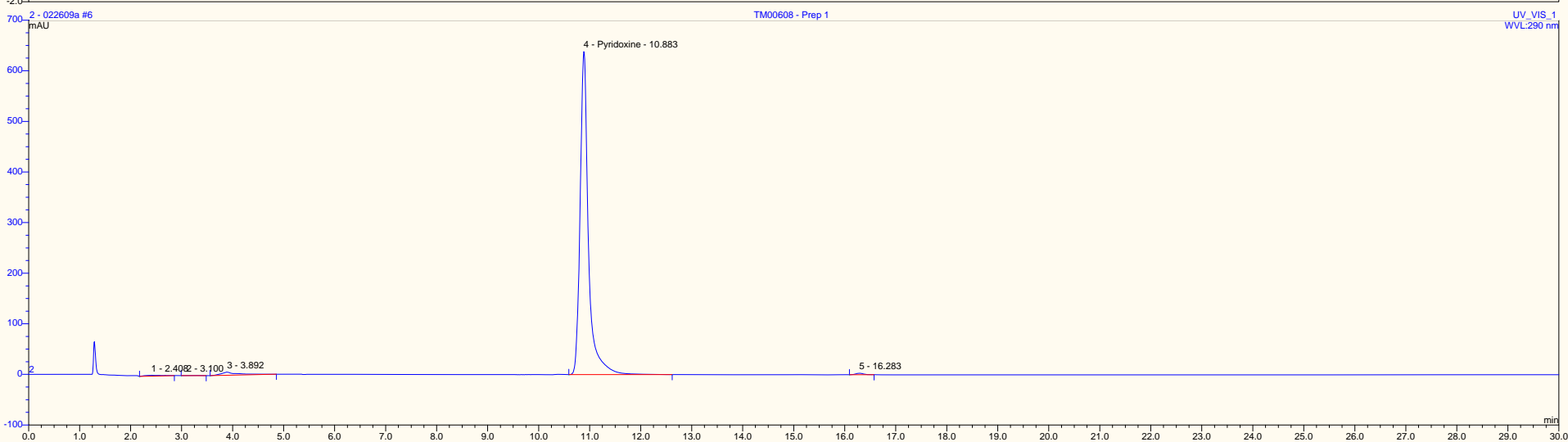
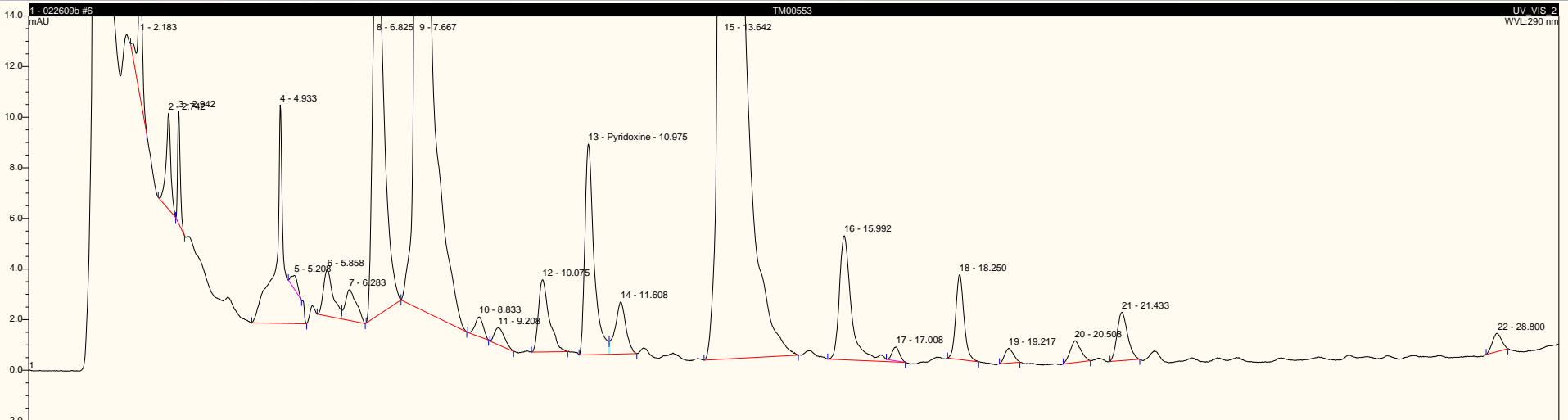


Challenges in Validation

Selenium (as Hydrolyzed Protein Chelate)	200.00 mcg	286%
Copper (as Copper Gluconate)	2.00 mg	100%
Manganese (as Manganese Sulfate)	2.00 mg	100%
Chromium (as Hydrolyzed Protein Chelate)	120.00 mcg	100%
Molybdenum (as Sodium Molybdate)	75.00 mcg	100%
Proprietary Sports Blend	215.00 mg	**
Green Tea Leaves Extract (Camellia sinensis)		**
Ginkgo Biloba Leaf Extract		**
L-Arginine		**
L-Carnitine		**
L-Glutamine		**
L-Taurine Ethyl Ester HCL		**
Branched Chain Amino Acid Blend	150.00 mg	**
L-Valine		**
L-Leucine		**
L-Isoleucine		**
alpha-Lipoic Acid	25.00 mg	**
Choline (as Choline Bitartrate)	10.00 mg	**
Inositol	10.00 mg	**
Silica (as Silicon Dioxide)	4.00 mg	**
Boron (as Hydrolyzed Protein Chelate)	2.00 mg	**
Lutein (as lutein esters)	950.00 mcg	**
Lycopene	950.00 mcg	**
Astaxanthin	50.00 mcg	**
Zeaxanthin	50.00 mcg	**
Vanadium (as Sodium Metavanadate)	10.00 mcg	**



Challenges in Validation





Challenges in Validation

- Simply using an AOAC method or USP method on your unique proprietary product is not enough.
- Need to demonstrate that the method is applicable to your product in your laboratory.





Fitness for Purpose

- Define the “Fitness for Purpose” of the method:
 - What are the analytes?
 - What are the matrices?
 - What are the expected concentration ranges?
 - Does the method need to be qualitative or quantitative (or both)?
 - Does the method serve any regulatory purpose?



Fitness for Purpose

- Intended application
 - What is the purpose of the method?
 - Stability testing
 - Research & Development
 - Process uniformity
 - Detection of adulterants or pesticides
 - Etc.
 - How often will the method be used?



Fitness for Purpose

- What setting will the method be used?
 - Manufacturer
 - Contract Testing Laboratory
 - Regulatory Agency
 - University



Fitness for Purpose

- The purpose of a method dictates how the method is developed and the validation requirements for the method.
 - A method designed to measure calcium levels in plasma is probably not suitable for assaying calcium carbonate raw material used in a dietary supplement, and vice-versa.
 - Different analyte concentrations
 - Different matrices
 - Different preparation of test solutions



Fitness for Purpose

- For assay methods (e.g. glucosamine in glucosamine HCl raw material), Limit of Detection/Limit of Quantitation is probably of little interest.
- For contaminant/residue methods, LOD/LOQ is usually of great importance.
 - Required LOD/LOQ may be specified by regulations.



Fitness for Purpose

- Define the Analytes
 - Dependant upon:
 - Label claims
 - Regulatory requirements
 - Safety/efficacy issues
 - Marketing Strategy
 - Individual Compounds vs. Class of Compounds
 - e.g. Heavy metals vs. lead
 - Specific form of analyte
 - E.g. Chromium III vs. Chromium VI



Fitness for Purpose - Challenges

- Often poorly defined analytes
 - “Flavonoids”
 - “Phenolics”
 - “Anti-oxidants”

- Define the Matrices
 - Multiple complex matrices
 - Raw Materials
 - Extracts
 - Tablets
 - Capsules
 - Softgels
 - Drink Mixes



Fitness for Purpose

- Method development and validation should cover all matrices needed.
- Goals of the method may be different for raw materials and finished products.
- **Define Concentration Range**
 - Specify upper and lower limits of quantitation needed.
 - Concentration range is concentration in matrix, not test solution.

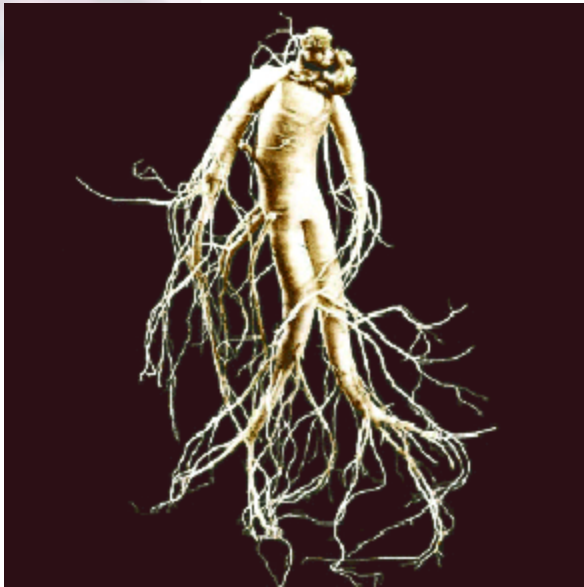


Fitness for Purpose

- Other Considerations:
 - Known potential interferences?
 - Accuracy/precision requirements?



Fitness for Purpose



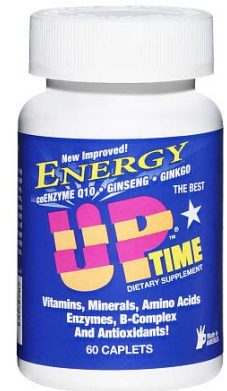
Root



Root Powder



Powdered Extract



Finished Products (?)

Testing of Ginsenosides in Panax Ginseng

Fitness for Purpose



Supplement Facts

Serving Size 3 Caplet(s)
Servings Per Container 20

Amount Per Serving		% DV
Vitamin C	900.00 mg	1500%
Calcium	700.00 mg	70%
Chromium	10.00 mcg	8%
Uptime power base	750.00 mg	**
Spirulina Blue Green Algae		**
wheat grass juice		**
alfalfa leaf		**
Caffeine		**
Papaya (fruit) Silica		**
Cayenne Pepper (fruit)		**
Siberian Ginseng extract		**
Echinacea (herb)		**
Ginkgo Biloba Extract		**
Carnitine tartrate		**
Coenzyme Q10		**
Chromium (dinicotinate)		**

** Daily Value (DV) not established

“Siberian Ginseng” (Eleuthero) contains eleutheroside, not ginsenosides!



Fitness for Purpose

- Challenges for 3rd Party and Regulatory Labs:
 - Don't know the quality of individual ingredients in finished products.
 - Need methods that can handle wide variety of products
 - Different matrices
 - Different “actives”
 - Different carriers or dosage forms
 - Different concentration ranges



Fitness for Purpose

- Validating methods for every product becomes onerous and impractical.
- How can we ensure that a method is “fit for purpose” on a product we’ve never tested before?



Fitness for Purpose

- Dry Lab
 - Cheap
 - No solvents used
 - No reference standards needed
 - No expensive instrumentation
 - Fast
 - Usually turn around a sample in <3 days
 - Always get the results you want!
 - Random number generators can have limits set so that result will always meet specification.

ANALYTICAL REPORT

TO:

DATE: June 14, 2006

Sample: Thyroid Support (60 capsules)	Lot Number: 110000
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Analyte	Result	Unit	Label
Vitamin A (as retinyl acetate)	2620 ± 22.5	IU / 2 cap	2500
Ascorbic acid	161.3 ± 1.59	mg / 2 cap	150
Vitamin D ₃ (cholecalciferol)	218.4 ± 1.96	IU / 2 cap	200
Kelp (calc. as 0.5% iodine)	40.53 ± 0.55	mg / 2 cap	30
Zinc (as citrate)	23.16 ± 0.16	mg / 2 cap	20
Selenium (as selenomethionine)	217.3 ± 2.44	mcg / 2 cap	200
L-Tyrosine	509.6 ± 4.15	mg / 2 cap	500
Ashwagandha (calc. as 5% withanolides)	466.3 ± 4.55	mg / 2 cap	400
Coleus forskohlii (calc. as 10% forskolin)	112.3 ± 1.27	mg / 2 cap	100
Guggul (calc. as 2.5% guggulsterones)	164.2 ± 1.66	mg / 2 cap	150
Curcuma (calc. as 97% curcuminoids)	108.3 ± 1.23	mg / 2 cap	100

Analysis performed on a composite sample made from ten capsules taken randomly from a sealed bottle of Thyroid Support consisting of 60 capsules received directly from Gamma Nutrition, Inc.; Capsules – hard clear VegiCap size 00, filled with a deep yellow flowing powder.
Average capsule fill weight = 791.1 mg

Retinyl analysis performed using HPLC by method adapted from Steghens, J.P., vanKappel, A.L., Riboli, E., Collombel, C., "Simultaneous Measurement of Seven Carotenoids, Retinol and Alpha-tocopherol by High-Performance Liquid Chromatography," as published in the Journal of Chromatography, B: Biomedical Applications, 694: 71-81, 1997; utilizing a sample mixed 0.2 ml ethanol then shaken for 5 min. H₂O (0.2 ml) and hexane (0.5 ml) were added and shaken for 5 min, and the organic phase separated. After second extraction with 0.3 ml hexane, combined extract was evaporated in vacuo and residue was dissolved in 0.3 ml hexane/ethanol/methanol (1:5:44). This solution was analyzed on two columns of 3 µm Adsorbosphere HS C18 (100x4.6 mm and 150x4.6 mm in series) at 37°C. The mobile phase (0.9 ml/min) was methanol/acetonitrile (2:3), containing 0.5% acetic acid for 7.1 min, then a step gradient to 24% of CH₂Cl₂ in the same solvent for 10.3 min, with detection at 292, 325, 450 and 473 nm. Ascorbic acid anion analysis performed using HPLC by method adapted from Castro RN, Azeredo LC, Azeredo MAA, de Sampaio CST, "HPLC Assay for the Determination of Ascorbic Acid in Honey Samples," as published in Journal Of Liquid Chromatography & Related Technologies 24 (7): 1015-1020 2001; utilizing a C-18-ODS column with an isocratic mobile phase consisting of a mixture of 15% methanol and 85% water, adjusted to pH 2.5 with metaphosphoric acid, at a flow



Fitness for Purpose

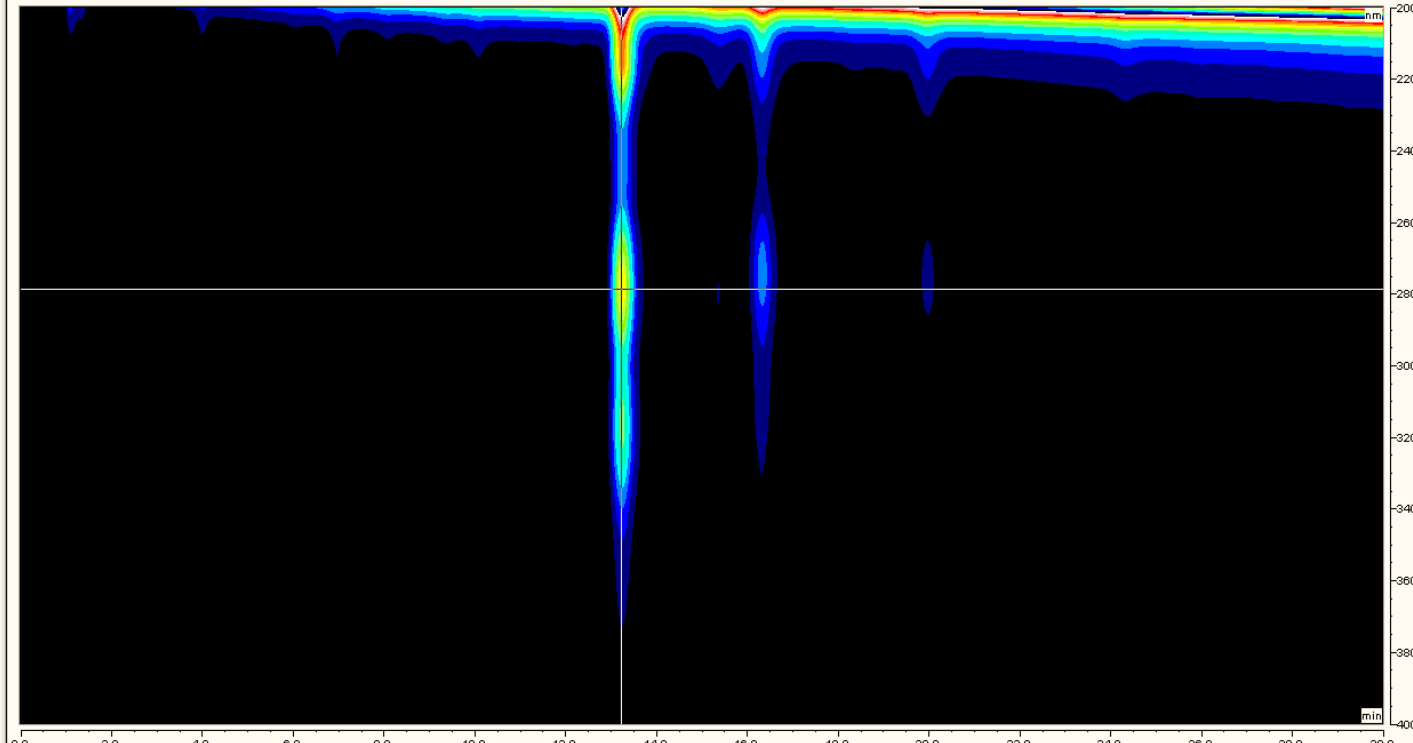
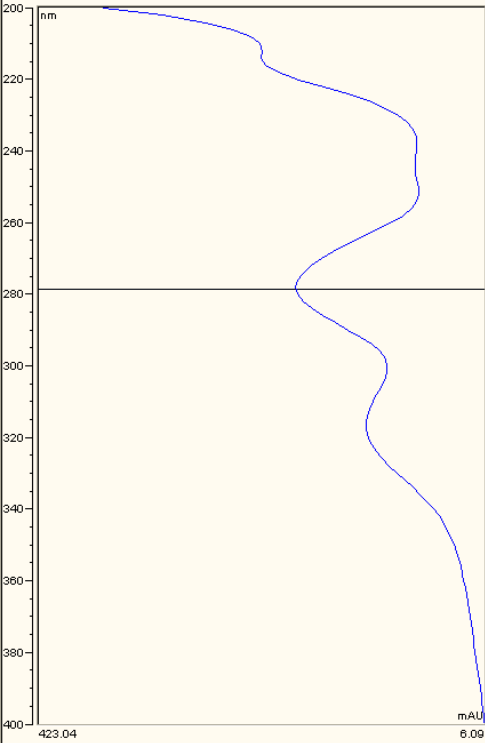
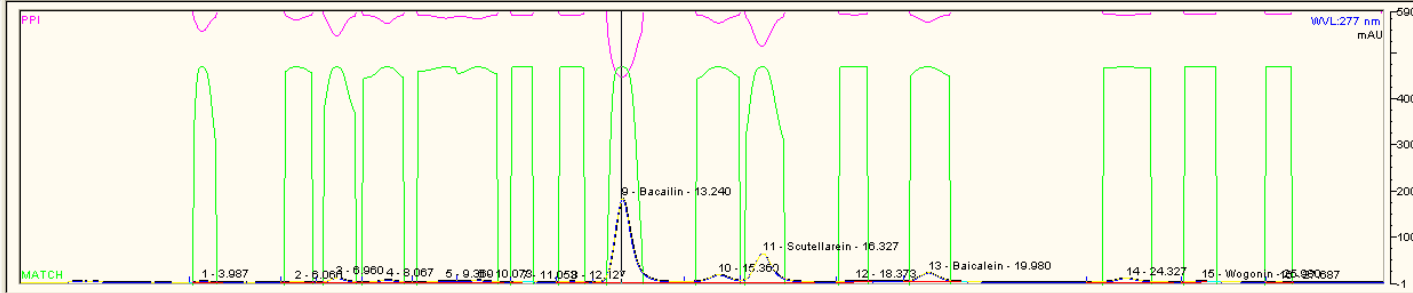
- Verify calibration range of method, and extend if necessary.
 - The *validated* calibration range of an AOAC method is not always the possible linear range of the method.
- For complex samples with many (often unknown) constituents, use PDA to ensure peak purity.

Fitness for Purpose

Chromleon - DCQ69VB1_localSequences\mromanSep07\091107test #9 Skullcap Extract - [DCQ69VB1_localSequences\mromanSep07\091107test #9 Skullcap Extract]

File Edit View Workspace Qualification Window Help

Ref.Chl.: UV_VIS_1
Name: 9:Skullcap Extract
Type: unknown blank
Status: finished
Date: 9/12/2007 12:56:20 PM
Pos/Vol: RA2/10.00
Dil/Fac: 1.00
Amount: 1.00
Weight: 1.00
Comment:



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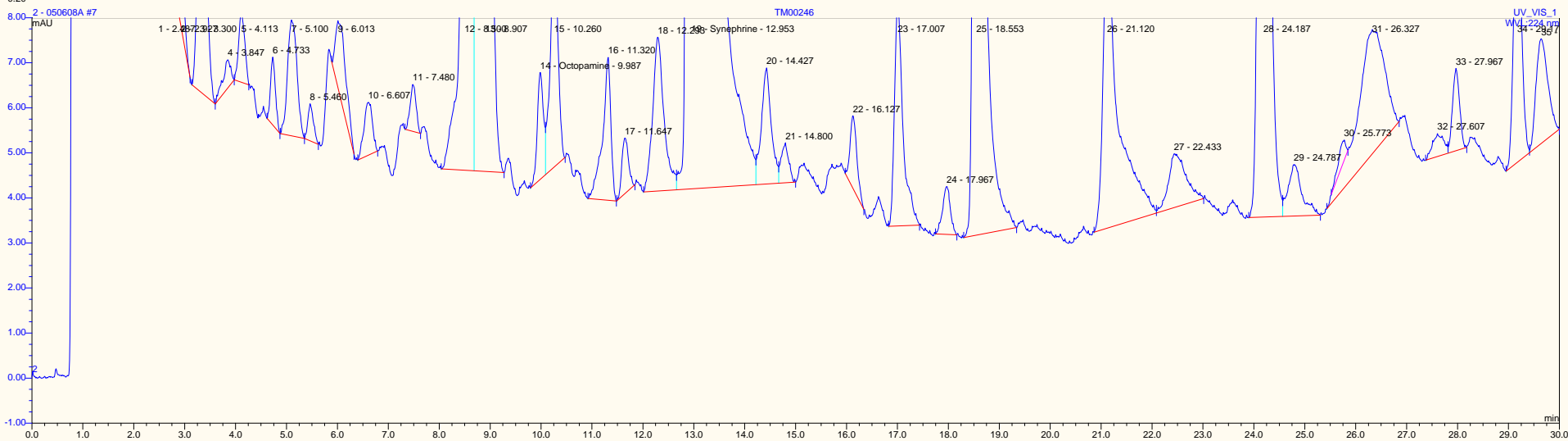
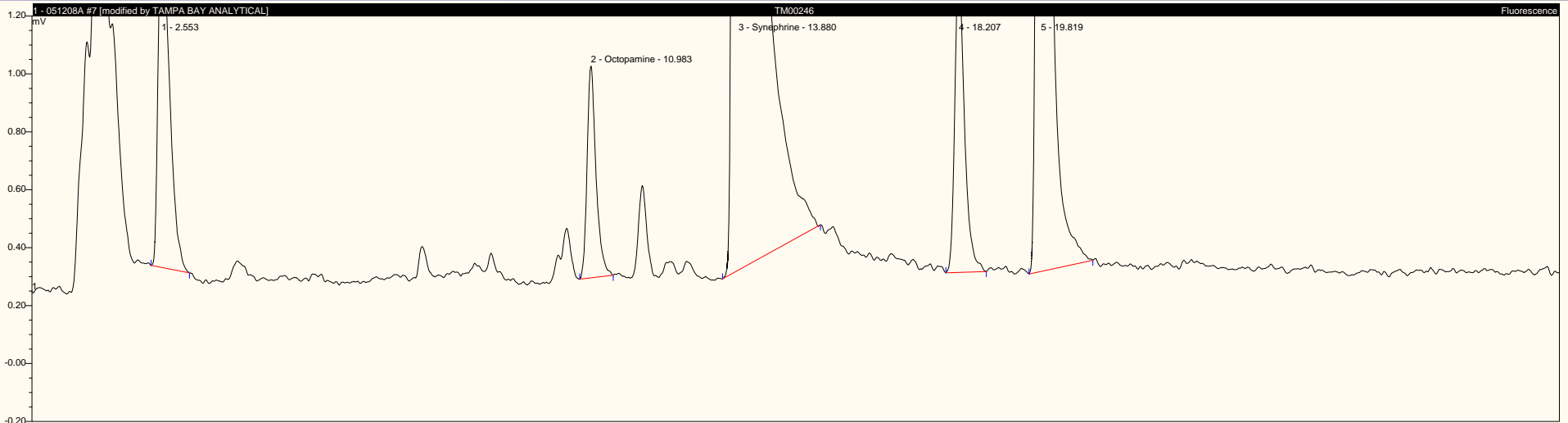


Fitness for Purpose

- Make replicate test preparations
 - Helps verify precision of method
 - Going from 1 test preparation to 2 test preparations reduces uncertainty by factor of 1.4.
- Perform spike recovery if you obtain anomalous results on new sample.
- Explore alternate detection techniques for samples that are very “dirty,” or have analytes below concentration range of method.
 - Can improve sensitivity and/or selectivity



Fitness for Purpose





Fitness for Purpose - Summary

- 1. Develop fitness for purpose statement.
 - Analytes
 - Matrices
 - Concentration Ranges
- 2. Develop a method to analyze all the target analytes in all the matrices at the expected concentration ranges.
- 3. Design a validation protocol to demonstrate that the candidate method fulfills the fitness for purpose statement.
- 4. Evaluate whether the validation data demonstrates that the method is suitable for its intended purpose.



Fitness for Purpose - Summary

- Official validated methods (e.g. AOAC, USP) may not be appropriate for all products.
 - Need to verify the fitness for purpose.
- Performing complete SLVs on each product is usually impossible for 3rd party labs.
- Confirming precision and selectivity helps demonstrate that a method is appropriate for a new product.
- Unusual results should be investigated.