

Bursting the Analytical Bubble

And you thought when you learned the truth about Santa Claus it ruined your day. Read on. . . .

Practitioners, retailers, and the public are currently suffering through an unrelenting barrage of claims and certifications from companies who claim they meet “cGMP” (current Good Manufacturing Practices) and all their products are “independently” tested. It makes for great press, and the official “independent” analyses look very impressive and oh-so reliable. I have previously opined on the desirability of requiring the laboratories that ostensibly analyze the ingredients in dietary supplements to undergo some version of CLIA certification, similar to what blood-testing laboratories are licensed under. Furthermore, even though FDA will soon publish its final rule that will impose GMPs on the dietary supplement industry, the laboratory analytical issue is not going to go away for decades.

Even the FDA in the proposed GMP rulemaking (pertinent parts from the Federal Register follow this editorial) acknowledges:

“We recognize that certain tests...for certain finished products may not be available due to complex finished matrices that would make such testing impracticable.” 68 Federal Register 12157, at 12197 (March 13, 2003)

Of course, you’re probably thinking the FDA was referring to exotic, heretofore unknown herbal combinations from previously undiscovered tropical islands. But you would only be half right. Because not only is it impossible to accurately analyze many combination products that contain herbal extracts, you also have about the same likelihood of obtaining an accurate, validated analysis of a number of extremely common nutrients as someone seeking an analysis of a municipal sewage plant sample to determine the number of citizens who ate M&M’s on Tuesday. In the FDA’s proposed GMPs, not only does FDA expressly acknowledge this analytical shortcoming (sans sewage plant reference), the agency suggests the solution is to test raw materials and perform in-process testing as products are being manufactured.

To demonstrate that this dilemma is not just a problem of analyzing a product with multiple herbs, I requested a pharmacist, who practices in Oregon, to submit for analysis a multiple vitamin/mineral product that was manufactured by one of the companies that utilizes as a primary sales tool the claim that all of their products undergo “independent” analysis. Capsules of their product were submitted with a request to analyze three ingredients: biotin, vitamin D3, and pyridoxal 5’-phosphate. The request asked for two different products to be tested (with the fake names of Daily Nutrient and Super Nutrient), although *both submissions actually came from a single bottle of product, which already had stated levels of these nutrients analyzed and reported by the same laboratory* (done in “six-capsules-contain” format). It was not revealed to the laboratory that the product being tested – re-bottled into two test batches – was one they had previously tested for an existing client.

The results that came back on the two lots analyzed by the laboratory were quite revealing, to say the least. For both of the pharmacist's faux multis, the "independent" laboratory's test results were conveniently "close" to the fictitious levels indicated on submission of the samples – one was thirty percent under, the other thirty percent over, the laboratory's previously "tested" actual levels.

Daily Nutrient – Lot # MR040117

Ingredient	Stated in Pharmacist's Request	Analytical Results Reported 11/5/2004	Company's Analysis 8/18/2004	Company's Product Label
Biotin	540 mcg.	519.18 mcg.	804.9 mcg.	800 mcg.
Pyridoxal 5'-Phosphate	18 mg.	19.062 mg.	26.03 mg.	25 mg.
Vitamin D3	270 IU.	256.56 IU.	409.5 IU.	400 IU.

Super Nutrient – Lot # MR04225

Ingredient	Stated in Pharmacist's Request	Analytical Results Reported 11/5/2004	Company's Analysis 8/18/2004	Company's Product Label
Biotin	1080 mcg.	1027.2 mcg.	804.9 mcg.	800 mcg.
Pyridoxal 5'-Phosphate	36 mg.	36.456 mg.	26.03 mg.	25 mg.
Vitamin D3	540 IU.	505.32 IU.	409.5 IU.	400 IU.

The table above contains the fabricated amounts from the pharmacist's requested analysis, the results obtained from the laboratory's analysis of the pharmacist's submission, the laboratory's original analysis of the real manufacturer's identical ingredients, and the real manufacturer's label claim of the ingredients. The pharmacist tendered a single instruction to the laboratory: "Please analyze both multi products for the same ingredients. They are at different levels, as outlined."

Not only did the laboratory report three different results for each of the three nutrients – apparently determined by what each customer claimed was in the product – the laboratory analyzed levels for ingredients which are virtually impossible to determine in quantitative levels in a finished product matrix. Truthfully, the company that would rely on this analysis has virtually no idea if the finished product is manufactured correctly.

As the FDA goes forward with promulgation of GMPs for the dietary supplement industry, it will become necessary to not only conduct raw material testing, but also to validate batch record keeping, thus ensuring that ingredients which cannot be confirmed in a finished product are actually in the product. In the long term, as dietary supplement manufacturers are constrained to implement the federal GMPs, they will have no option but to invest in in-house laboratory equipment for ongoing validation testing. They will then have to be realistic and utilize proper analytical techniques within the actual limits of available *validated* methods.

Another problem area that hopefully will be eliminated by the institution of federal GMP guidelines is the practice of companies having their products “certified” by various for-profit groups in the health food area. Obviously, it’s very reassuring to a consumer to find somebody’s “seal of approval” on the label of a finished product, and I suspect the practice has probably improved product quality overall in the industry. Having admitted that, however, the product analyzed above had a “certification” on the label.

Practitioners and consumers need to become savvy enough to smell a rotten egg when one is handed to them. In many cases, the analysis presented is too “perfect” and does not correspond to the actual, and acceptable, margin of error, which is inherent in every validated procedure. In my opinion, the above analysis constitutes blatant fraud, and any company that relies on this laboratory should immediately discontinue any association with them.

The following list of some common ingredients, prepared in conjunction with ChromaDex (the company that supplies analytical standards and performs some analysis for the USP) is broken down into categories based on their individual analytical difficulty, or their difficulty to analyze when mixed into a matrix of other ingredients. When you see an independent analysis of a product containing the multiple ingredients marked “impossible” on this list, you would be best advised to pass on the product. Santa did.

Al Czap
Publisher

The following common ingredients are a partial listing of materials and their current analytical limitations.

Table 1. Identity and potency in finished product can be accurately tested for

Acetyl-L-Carnitine	Hydrastis canadensis (goldenseal)
N-Acetyl-L-Cysteine	5-Hydroxytryptophan
L-Alanine	Hypericum perforatum (St. John's wort)
Alpha ketoglutaric acid	Indole-3-Carbinol
Alpha Lipoic Acid	Iodine
Angelica sinensis (dong quai)	Ipriflavone
L-Arginine	Iron
Ascorbic acid (vitamin C)	L-Isoleucine
(Can be difficult depending on matrix)	L-Leucine
Ascorbyl Palmitate	Lutein (Still working on optimizing method)
(Can be difficult depending on matrix)	Lycopene (Still working on optimizing method)
Bacopa monniera (brahmi)	L-Lysine
(Method is still under development)	Magnesium
Berberis aquifolium (Oregon grape)	Malic Acid (Can be difficult depending on matrix)
(Many methods for many analytes)	Manganese
Bioflavonoids (citrus)	Matricaria chamomilla
Black Currant Oil	Melatonin
Boswellia serrata (frankincense)	L-Methionine
Butcher's broom (Ruscus aculeatus)	Molybdenum
(Can be difficult depending on matrix)	MSM (Methyl Sulfonyl Methane)
Calcium	Niacin (vitamin B3)
Calcium d-Glucarate	Niacinamide
Calcium pantothenate (vitamin B5)	Oligomeric proanthocyanidins extract (pycnogenols)
(Can be difficult depending on matrix)	(Can be difficult depending on matrix)
L-Carnitine (Can be difficult depending on matrix)	Olive leaf
Cassia acutifolia (senna)	Panax ginseng (Oriental ginseng)
Centella asiatica (Gotu Kola)	Passiflora incarnata (passion flower)
(Can be difficult depending on matrix)	DL-Phenylalanine (DLPA)
Chamomile (see Matricaria chamomilla)	Phosphatidylcholine (Can be difficult depending on matrix)
Chaste Tree (see Vitex agnus castus)	Phosphatidylserine (Can be difficult depending on matrix)
Cholecalciferol (vitamin D3)	Picrorhiza kurroa
Chondroitin sulfate	Piper longa
Chromium	Potassium
Cimicifuga racemosa (black cohosh)	L-Proline
(Still working on optimizing method)	Prunus africanus (pygeum)
Co-enzyme Q10	Pyridoxine HCl (vitamin B6)
Copper	Red Yeast Rice
Crataegus oxycantha (hawthorne)	Rhodiola rosea
Curcuma longa (turmeric)	Riboflavin (vitamin B2)
Cynara scolymus (artichoke)	R-Lipoic Acid
(Can be difficult depending on matrix)	Selenium
L-Cysteine	Serenoa repens (saw palmetto)
DHEA (dehydroepiandrosterone)	L-Serine
Diosmin	Siberian Ginseng
DMSA (meso-2,3-dimercaptosuccinic acid)	Silybum marianum (milk thistle)
(Still working on optimizing method)	Soy Isoflavones
Docosahexaenoic acid (DHA)	Taurine
Echinacea angustifolia	Thiamine HCl (vitamin B1)
Eicosapentaenoic acid (EPA)	L-Threonine
Eleuthero extract (Eleutherococcus senticosus)	L-Tyrosine
Gamma linolenic acid (GLA)	Uva ursi (bearberry)
Ginkgo biloba (Terpene lactones can be difficult)	Vaccinium myrtillus (bilberry)
Glucosamine sulfate	Valeriana officinalis (valerian)
L-Glutamic acid	L-Valine
L-Glutamine	Vanadium
L-Glutathione	Vanadyl sulfate
Glycine	Viburnum opulus (Cramp Bark)
Gotu Kola (Centella asiatica)	Vinpocetine
(Can be difficult depending on matrix)	Vitamin A (palmitate)
Green tea extract	Vitamin K1
Gymnema sylvestre	Vitamin K2
Harpagophytum procumbens (devil's claw)	Vitex agnus castus (chaste tree)
Hesperidin methyl chalcone	Withania somnifera (ashwagandha)
L-Histidine	Zinc
Humulus lupulus (hops)	Zingiber officinalis (ginger)

FDA Proposed GMPs

[68 *Federal Register* 49:12157, at 12197-12198; March 13, 2003]

“For those specifications that we tentatively have determined are necessary (identity, purity, quality, strength, and composition) at receipt, in process, and finished product stage, we are proposing specific testing requirements that provide some flexibility. Under Sec. 111.35(g)(1), we would require that you test each finished batch of the dietary ingredient or dietary supplement produced before releasing for distribution to confirm that specifications are met for the identity, purity, quality, strength, and composition intended, provided that there are scientifically valid analytical methods available to perform such testing. We recognize that certain tests for identity, purity, quality, strength, or composition for certain finished product may not be available due to complex finished matrices that would make such testing impracticable. Further, even though there may not be a scientifically valid analytical method that you could use to provide you with the information to evaluate, for example, the identity and composition of the finished product, there may be methods available for testing at the finished product stage for other required specifications of purity, quality, and strength. Under proposed Sec. 111.35(g)(3), your quality control must document that a scientifically valid analytical method is not available to perform finished product testing for any one of the required specifications for identity, purity, quality, strength or composition.

Table 2. Identity and potency only as raw material can be accurately tested for, not in a finished product matrix

Adenosylcobalamin (Difficult)
 Beta Carotene
 (Methods are still being developed for various matrix)
 Biotin (Not valid for finished dosage)
 Cyanocobalamin (vitamin B12)
 d-alpha-Tocopherol
 d-alpha-Tocopheryl
 Folic acid
 Folinic acid
 Inositol (Can be difficult depending on matrix)
 Inositol hexaniacinate (Can be difficult depending on matrix)
 Methylcobalamin
 Mixed Carotenes
 Mixed Tocopherols
 Pyridoxal 5'-phosphate
 Riboflavin 5'-phosphate

Table 3. Identity only as a raw material can be accurately tested for, potency cannot be accurately tested as an individual raw material, or in a matrix

Buchu (*Barosma betulina*)
 Buckthorne (*Rhamnus frangula*)
 Burdock (*Arctium lappa*)
 Chelidonium majus (celandine)
 Hamamelis virginiana (witch hazel)
 Hydrangea arborescens (seven barks)
 Iris versicolor (blue flag)
 Isatis tinctoria (Dyer's woad)
 Medicago sativa (alfalfa) (Working on standards)
 Paeonia suffruticosa (tree peony)
 Rhamnus frangula (buckthorne)
 Rhamnus purshiana (cascara)
 Taraxacum officinale (dandelion root) (Working on standards)
 Tylophora asthmatica
 Urtica dioica (stinging nettle)

“If your quality control unit documents that a scientifically valid analytical method for testing each batch of dietary ingredient or dietary supplement is not available for any one of those required specifications, then you would be required, under Sec. 111.35(g)(2)(i) and (g)(2)(ii) to test incoming shipment lots of components, dietary ingredients, or dietary supplements for any such specification to determine whether it is met and to test in-process for any such specification in accordance with the master manufacturing record where control is necessary to ensure the identity, purity, quality, strength, and composition of dietary ingredients or dietary supplements.

...

“If you are able to perform testing on each finished batch of dietary ingredient or dietary supplement to confirm that specifications are met for the identity, purity, quality, strength, and composition intended, then we would recommend, but would not require, that you also test materials received for these same specifications to ensure that they are the right ingredients and so that you do not end up having to destroy an entire batch of finished product after using an erroneous ingredient that could have otherwise been identified earlier before being added to a batch.

...

“Proposed Sec. 111.35(h) would require that you use an appropriate test or examination to determine whether your specifications are met. An appropriate test is one that is a scientifically valid analytical method. If there is an AOAC or FDA method available that is appropriate for your purpose, you should use that test method. For example, if your dietary supplement claims to contain vitamin C, there is a specific test for identifying vitamin C, and so proposed Sec. 111.35(h) would require that you use that test (Ref. 68). If an AOAC or FDA method is not available, a scientifically valid analytical method is one that is based on scientific data or results published in, for example, scientific journals, references, textbooks, or proprietary research. While there may not be an AOAC or FDA method available, we are not aware of a situation where an appropriate scientifically valid analytical method is not available. You could perform the tests yourself or have someone perform these tests for you.”

[68 *Federal Register* 49:12157, at 12207; March 13, 2003]

“One comment to the ANPRM recommended that the regulations related to laboratory operations apply to laboratory facilities located and operated within a company and those facilities that a company may contract with that are located elsewhere. Proposed Sec. 111.60(a) would apply to laboratory facilities generally and is not restricted to laboratory facilities located and operated within a company. In other words, even if you hire a private laboratory to perform various tests for you, proposed Sec. 111.60(a) would require that you make sure that the private laboratory’s facilities are adequate to perform whatever tests are necessary. The most important point in proposed Sec. 111.60(a), however, is not where the facility is located, but whether the laboratory facility is adequate for the tests and examinations that need to be done.

[68 *Federal Register* 49:12157, at 12208-12209; March 13, 2003]

“Proposed Sec. 111.60(b)(iv) through (b)(vi) would require . . . Use of appropriate test method validations. Test method validation determines whether a newly-developed or existing test method is accurate, precise, and specific for its intended purpose. We have discussed previously the terms “accurate” and “precise.” Validation involves evaluating the test method on multiple occasions or in multiple test facilities. Official methods, such as AOAC International methods, are validated in collaborative studies using several laboratories under identical conditions. The AOAC International methods that are validated in collaborative studies are often cited as “official validated methods.”

. . .

“Use of test methods in accordance with established criteria. Your process for performing test methods criteria must include sufficient detail, including the material you are testing, the purpose of the test, and the test method. The description of the test method criteria must include any reagents used and preparation instructions, apparatus required, any instructions for preparing the sample to be tested, and instructions for conducting the examination.

. . .

“Your test methods criteria must specify the component, dietary ingredient, or dietary supplement to be tested, and what specifically to test for, e.g., the identity of the component, dietary ingredient, or dietary supplement, or a specific contaminant. The method criteria must provide detailed information about performing the analysis (i.e., the reagent solutions needed and their preparation, the type of microscope and other equipment required, preparing the sample, and examination instructions). The proposed rule would not require that you test for any specific substance and would not require a specific test for a substance, so you would be able to evaluate what the most appropriate test would be for the component, dietary ingredient, or dietary supplement and to use the test methods that are suited to your products and your manufacturing needs. Your test methodology must be specific for the component, dietary ingredient, or dietary supplement and the specifications you have established.

. . .

“Proposed Sec. 111.60(c) would require that you verify that the laboratory testing methodologies are appropriate for their intended use.

“Proposed Sec. 111.60(d) would require that you identify and use the appropriate validated testing method to use for each established specification for which testing is required to determine whether the specification is met. In other words, the proposal would recognize that requiring that you have testing methods is not sufficient alone; you must also use those testing methods to prevent the adulteration of dietary ingredients or dietary supplements.”