Adulteration: Spotlight on Bilberry

With adulteration present in the marketplace, it is important that companies know how to spot the problem quickly and easily.

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By now the industry should be well aware of the importance of reliable testing for dietary supplement ingredients and finished products. While federal regulations may place the burden of testing on various parties, supplier on down, there is a responsibility for companies in the trade to ascertain the identity of the materials that they handle. Expediency requires that quality need not be assured by redundant testing, and it's not necessary to continue to verify what may already be known, but mechanisms must exist whereby the trade assures the quality and identity of marketplace materials.

Much has been said and written about product and ingredient testing, with proposals for various seals of approval, or disapproval in some cases, receiving considerable attention—as they well should. However, there is something lacking in that approach, something that takes responsibility out of the hands of those who should exercise it. When someone else has specialized knowledge that your business may depend on, and you don't understand that field or are otherwise unable to meaningfully participate in it, there is a problem.

This article in a nutshell:
- Quality problems surfacing in many markets
- Bilberry adulteration
- Problems, tools, solutions
- Conclusion

Quality Problems Surfacing in Many Markets

Quality is nothing if it is not assured, and identity is sometimes the first quality "masquerade" when ingredients are intentionally adulterated. Low price for otherwise valued ingredients is the proverbial red flag for potential low quality, adulterated, substituted, or otherwise improper materials and products.

Adulteration driven by economic factors may have health risks. This issue hits home, especially when one considers the very recent melamine pet food debacle, where a material was added to an ingredient in a way that it would not be detected by the usual assay. By all appearances, this material was added as a cheap way to meet what are now obviously inadequate specifications. Compounding this ingredient misrepresentation and tampering was the fact that others down the supply line did not conduct proper identification tests, if any.

This has a familiar ring to recent cases of poisoning by diethylene glycol (DEG) sold as glycerin. This substitution has historical significance in the U.S., as the use of DEG in a liquid drug preparation resulted in the deaths of 107 people, which prompted the passage of the Federal Food, Drug, and Cosmetic Act, requiring pre-market demonstration of safety for new drugs. The current situation, with poisoning outbreaks in Argentina, Bangladesh, Haiti, India, Nigeria, and most recently Panama, has resulted in the deaths of hundreds of children.

An investigative *New York Times* article on DEG revealed the same story as melamine—deliberate adulteration or substitution driven by economic gain and tacitly supported by suppliers without verified ingredient identity or quality. In this case, certificates of analysis (CoAs) were copied uncritically as if the goal was to sell a product regardless of what it actually was. The regulatory response in the U.S. was to inform industry of the problem, and ask it to test or adequately ensure that every container of every lot was tested for DEG contamination by a reputable supplier; the testing methodology used to make this determination was also mentioned.

In this case, no seals of approval were offered and no one appears to be testing the glycerin supply with a tell-all promise for a paid subscription. No. The problem was brought to the attention of industry, industry was reminded of its responsibility to produce safe products, and the tools to determine what is safe were provided—problem, tools, solution. These are simple non-binding recommendations.

Melamine contaminated pet food, adulterated glycerin, inadequate specifications, reliance on questionable CoAs—sound familiar? Dietary Supplement GMPs will require identity testing and verification of the adequacy and/or reliability of CoAs. This is understandable considering the raft of recent food and drug issues (melamine adulteration of gluten to fool the protein assay, and DEG contaminated glycerin, respectively). But this industry should not be concerned whether or not this can happen with supplement ingredients—it already has.

Bilberry Adulteration

Currently the dietary supplement industry is witnessing the adulteration and/or outright substitution of bilberry (*Vaccinium myrtillus*) extracts with other material fortified with a chemical dye called amaranth. This color is not from the South American amaranth plant, the flowers of which produce a deep red dye. That natural dye gave way to a single chemical coal tar-derived compound named amaranth that is more commonly known as Red Dye No. 2. Most adults today may remember Red Dye No. 2 from coloring baking kits they used as kids—they probably used it to color cupcake frosting and decorative Easter eggs. But children in the U.S. today don't get the opportunity to play with Red Dye No. 2 because FDA banned it from the food supply as a suspected carcinogen in 1976. This is, of course, unless they come across a sample of fake bilberry extract, which currently appears to be readily available at the bargain price of one-third off in the supplement marketplace.

At least one company conducting identity tests on bilberry sources noticed that some less expensive materials, while passing a general test for the 25% anthocyanin standard, did not produce a characteristic bilberry pattern of individual anthocyanins upon further examination. The published report (*Penman et al. J. Agric. Food Chem 2006;54(19):7378-7382.*) found that two different extracts produced an adequate response to qualify for a 25% anthocyanin claim when examined by a method that essentially measured the intensity of the color present. This is an adequate assay so long as one has the genuine article. It was shown to be inadequate, however, when dealing with intentionally adulterated material.

Using a method that reveals the presence of 15 individual anthocyanins that make up 25% of bilberry extract, the researchers noticed a difference between the relative ratios of these compounds compared to the total 25%. One extract had a profile characteristic of bilberry and the other did not. In fact, the other had an additional compound that didn't behave like an anthocyanin at all. Once isolated, researchers were able to confirm its identity as amaranth dye (i.e., Red No. 2.).

The composition of the anthocyanin content of the fake extract was not consistent with that of bilberry, making it more than likely that another anthocyanin-rich material was extracted and "supplemented" with enough amaranth dye to satisfy the 25% anthocyanin specification (just like the melamine added to gluten to meet a protein limit requirement in the pet food debacle)—keep in mind that this problem can only be identified if the purchasing company actually tests the material or otherwise assures that an appropriate test is done to verify that what they purchased performs to specification. And herein lies the problem for many in the dietary supplement industry.

Problem, Tools, Solution

The awareness of adulteration has reached a fever pitch, often leaving many companies no choice but to "leave it to the experts" to deal with. This "black box solution" can make

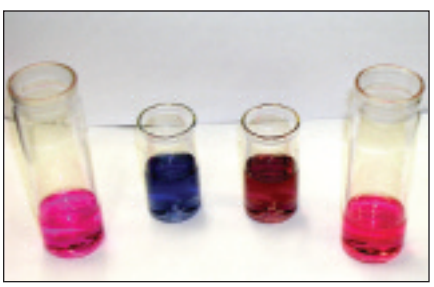
companies uncomfortable, because they may not want to turn over control to a third party to carry out their "scientific magic," especially since it may affect the bottom line.

The awareness of adulteration is widespread among industry companies, and a tool, albeit not very understandable to the non-scientist, has been identified. In this case, and in many others, there are simple tools that present a solution, both helping to elucidate the problem in the process and demonstrate a clearly understandable resolution.

This approach is similar that taken on the "Antiques Roadshow." For those not familiar with the show, *Wikipedia* explains it this way: "People bring along their possessions to be evaluated for authenticity and interest…and an approximate valuation is given." The evaluation is not a scientific one, but an expert one nevertheless. The most valuable item featured on the show was a 19th century Navajo blanket containing a small red cloth rectangle that helped date the blanket. The red dye used in this case was insect derived cochineal.

The point is, expert evaluations have their place and can correctly inform in the absence (or even the presence) of sophisticated validated assays—problem, tools, solution. One tool for solving the problem of bilberry adulteration involves evaluating actual diluted solutions (*see Image 1*).

IMAGE 1

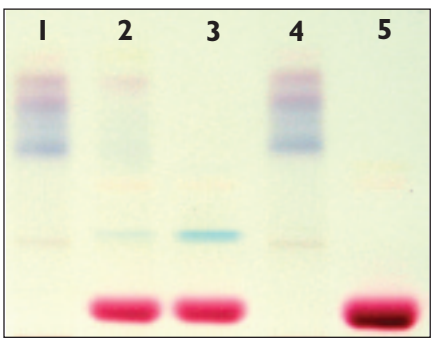


From Left to Right: *Very dilute bilberry, bilberry adjusted to pH>10, fake bilberry adjusted to pH>10, and fake, very dilute bilberry—Photo courtesy of MediHerb, Palmyra, WI*

The genuine bilberry extract of commerce is reddish in color when diluted in water. When the pH of that solution is raised above 10, a characteristic of anthocyanins comes into play—they turn blue as seen in the second tube from the left in *Image 1*. The third tube from the left is adulterated material under the same condition. It is not blue. Suffice it say, this simple tool is quite instructive!

Another simple tool is thin-layer chromatography (TLC), also known as HPTLC when using high performance thin-layer plates. Its classic application is the separation of color dyes. Take a look at *Image 2*. Lanes 1 and 4 pertain to genuine bilberry, lanes 2 and 3 expose the fakes, and lane 5 is the pure adulterant (amaranth dye). Note how the materials in lanes 2 and 3

IMAGE 2



From Left to Right: *Lane 1 genuine bilberry, lane 2 and 3 fake bilberry, lane 4 genuine bilberry, lane 5 pure adulterant (i.e., amaranth dye)—Photo courtesy of MediHerb, Palmyra, WI*

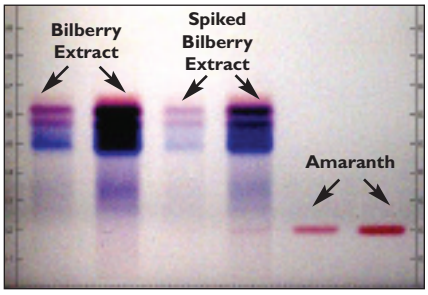
don't appear to match the bilberry standard in lanes 1 and 4, but how the presence of amaranth compared to lane 5 is obvious.

The distinction between genuine bilberry and the amaranth adulterant can also be seen in *Image 3*, which demonstrates results with HPTLC plates and enhanced light. This example shows bilberry extract adulterated with amaranth by a method that has been reported to be able to detect adulteration even at trace levels of 250 ppm. Genuine bilberry extract would likely not need to be "fortified" (a nice way to say illegally spiked) in this manner—only illegal inferior materials that appear to be available in today's marketplace.

Conclusion

The non-binding recommendation (and the absolute responsibility of the botanical trade) is to pay attention to the quality of not only bilberry extracts, but all herbal extracts from sources other than the known reputable suppliers. In this case, a 25% anthocyanin specification does not a bilberry extract make. Simple test methods are available that both illustrate the problem and provide a solution. Standards and laboratories qualified to do the analyses are easy to find and are absolutely necessary given the questionable quality of some materials in today's marketplace. NW

IMAGE 3



From Left to Right: *Genuine bilberry extract, spiked bilberry extract, amaranth—photo courtesy of CAMAG, Wilmington, NC*

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