Nutritional Outlook

THE MAGAZINE FOR THE FORMULATION, PRODUCTION, & DISTRIBUTION OF NUTRITIONAL PRODUCTS

Inaccurate Analytical Results

There are six major reasons why your test results may not match up.

By Joseph Zhou, Heidi Li, Ted Waszkuc, and Upasana Abbott

A company decides to test a particular vitamin, mineral, herb, or combination product. A sample is sent to a laboratory to test specific active ingredients. How accurate are the results?

We have seen dramatically different results from different laboratories testing the same product or ingredient. In tests run on a multivitamin that contained 5.0 mg of PABA (paraaminobenzoic acid), one lab found 16.6 mg per capsule, while another reported only 0.87 mg. In another test, samples that contained 83.0 mg of SAMe per tablet were sent to three separate labs. One lab found 108 mg but later revised its answer to 82.6 mg. Another found 128 mg but then revised its answer to 94.0 mg. A third lab found 106 mg and later revised its answer to 83.3 mg.

Incorrect test results mislead both consumers and manufacturers. If the actual potency is low but tests high, the product may be ineffective. On the other hand, if a product's actual potency is high but tests low, manufacturers will pay more to enhance potency, which may also carry health implications.

SIX DEGREES OF ESTIMATION

There are six major reasons why analytical results vary. The first is the analytical technique itself.

One of the older analytical methods, titrimetry uses a chemical reaction between the sample and a known volume of a reagent. This method is not selective because it will mistakenly count similar substances as the target active ingredient. It is a simple, economic analytical method, but yields less-reliable and often artificially high results.

Spectrophotometry (e.g., ultraviolet/visible (UV/VIS)) is a slightly more modern technique. The UV/VIS quantitation is based on the amount of light absorbed by the target active ingredient at a particular wavelength in the ultraviolet and visible spectrum. Like titrimetry, this method is also not particularly selective, often counting other ingredients with similar light-absorbing qualities. Results can be artificially high.

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> The top six reasons for inaccurate test results:
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> 1. Incorrect analytical technique
> 2. Poor quality-control standards
> 3. Poor extract
> 4. Decomposition of active ingredients
> 5. Human error
> 6. Variable definitions of active ingredients

Chromatography (e.g., high-pressure liquid chromatography (HPLC) or gas chromatography (GC)) separates the target active ingredient(s) from a solution by passing it through a sophisticated separation column combined with suitable mobile-phase or carry gas. Separated ingredients are then measured using UV or other detection methods. Development of HPLC or GC methods is a precise science, involving significant cost.

Even within HPLC or GC, if the peaks shown in the chromatograms are not well separated, interference can cause artificially high results. With milk thistle, UV/VIS usually reports 50–70% higher than HPLC for silymarin. With St. John's wort, UV/VIS typically reports 30–50% higher for hypericin. The differences are the result of a UV/VIS overcount due to interference.

POOR COMPARISON STANDARDS

The second major mistake is the use of poor-quality comparison standards. All of these analytical methods depend on having standard samples of known quality to use as a basis of comparison, a process called calibration in analytical science. These standards will lose some of their potency through improper storage.

When used in subsequent tests, the product being tested inaccurately seems more potent by comparison. This has been shown to be one of the common reasons for inaccurate test results for SAMe.

Using poor extract is the third major mistake. When preparing samples for HPLC or GC testing, the active ingredient often needs to be extracted from the sample. Using an incorrect solvent or failing to repeat the extraction process a sufficient number of times can result in not getting out all of the active ingredient, which leads to a false-low result.

The fourth major error is decomposition of the active ingredients during testing. Most of the active ingredients in natural products are not stable when extracted. For example, constant attention is necessary to keep the active ingredient in vitamin C stable throughout testing, including lowering temperatures during extraction and throughout the HPLC injection and separation.

Human error is the fifth major cause of testing inaccuracies. Careless or improperly trained laboratory personnel can produce unreliable results, and even seemingly good results need thoughtful review. A technician reported results of 9.6%, 9.6%, 9.9%, and 9.9% while performing single-extraction analyses, and 7.3%, 7.3%, 7.3%, and 7.3% while performing multiple-extraction analyses of the same active ingredient of the same sample. Although these seem very accurate and repeatable, multiple extraction analyses should yield higher results than a single extraction, calling the entire outcome into question.

Even seemingly good laboratory results need thoughtful review.

Finally, manufacturers sometimes differ in what they count as part of the active ingredient, such as determining how many compounds to include as part of the total phenols in echinacea or as isoflavones in soy extract. In general, the more compounds that are included, the higher the result. Also, in some UV/VIS analyses, predetermined conversion factors are used to replace a costly calibration process. These factors need to be verified and used cautiously or they will give incorrect results. ❖

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