

Public Health Impact

A Suitable Test for L-citrulline— How Validated Test Methods May Impact Public Health

In the U.S., most newborn babies are screened for rare inborn errors of metabolism (IEM)—a group of genetic disorders caused by a defect in a metabolic pathway. Left untreated, these disorders can lead to a host of medical and developmental consequences ranging from intellectual disability to severe cognitive impairment and even death.¹

Some of these metabolic errors require the use of amino acids to avoid the buildup of ammonia in the body. L-citrulline is one such amino acid, and it is used for IEM as a medical food, which is regulated by the U.S. Food and Drug Administration (FDA) under the Orphan Drug Act. [Medical foods are distinct within the broader category of foods](#) and need to be administered under the supervision of a healthcare professional specifically to help manage a disease or condition associated with distinctive nutritional requirements. In addition to being marketed as a medical food for IEM, L-citrulline is marketed as a dietary supplement for other purposes.

Sometimes, the quality control testing done to authenticate ingredients is insufficient to guarantee their quality. That was the case with L-citrulline in 2014.

One of the primary suppliers of the ingredient for use as a medical food voluntarily recalled several batches of the product after the [FDA warned that the product being sold had no L-citrulline present](#). The agency had received adverse event reports associated with L-citrulline and its lack of efficacy for patients, and conducted their own testing of the batches in question.

Despite the fact that a certificate of analysis (CoA) was provided by the supplier positively identifying the product as L-citrulline with an assay value of 99.88%, it was found that affected batches actually contained N-acetyl-leucine, another amino acid with a molecular weight and chemical structure similar to L-citrulline but not used in the treatment of IEM. The CoA provided did not specify the assay method used to authenticate the ingredient, but it is likely that the assay method used was a non-specific method such as acid-base titration, which would present similar readouts for both amino acids. A discriminating method, such as one based on High-Performance Liquid Chromatography (HPLC), potentially could have averted the mix-up between the amino acids and the resulting adverse events.

Upon learning about the L-citrulline recall, USP recognized the public health need for a scientifically validated test method that differentiated

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New Dietary Supplements Reference Standards

Below is a list of newly released reference standards:

Botanicals

- [2,3,5,4'-Tetrahydroxystilbebe-2-O-Beta-D-Glucoside](#)
- [Emodin](#)
- [Polygonum multiflorum Root Dry Extract](#)
- [Lonicera japonica Flower Dry Extract](#)
- [Secoxyloganin](#)
- [Lonicera macranthoides Flower Dry Extract](#)
- [Luteolin-7-O-glucoside](#)

Non-Botanicals

- [L-Citrulline](#)

Reference Standards Coming Soon

Botanicals

- Cinnamaldehyde
- *Cinnamomum cassia* Twig Powder
- Oleanolic Acid

Non-Botanicals

- Plant Stanol Esters
- Epicoprostanol

L-citrulline from N-acetyl-leucine, thus offering manufacturers a way to:

- Help ensure compliance with regulatory requirements.
- Test ingredients and products in a science-based manner.
- Help ensure quality ingredients and products, thus safeguarding their brands and protecting the health of users and patients.

The new USP monograph for L-citrulline is a dietary supplement monograph, and USP is exploring development of a similar L-citrulline monograph for inclusion in the *Food Chemicals Codex (FCC)*, which applies specifically to food ingredients. The new monograph includes an HPLC method that can discriminate L-citrulline from other closely related amino acids, namely N-acetyl-leucine and arginine. USP specifications also include a requirement for not less than 98.0% L-citrulline content, and a limit for related compounds at not more than 2.0%. Along with the test method, USP has developed a [reference standard for L-citrulline](#), which manufacturers can use to authenticate their ingredients and products.

For more information on USP's work in developing public standards for dietary supplements, visit <http://www.usp.org/dietary-supplements/overview>.

¹ Camp KM, Lloyd-Puryear MA, Huntington KL. Nutritional treatment for inborn errors of metabolism: indications, regulations, and availability of medical foods and dietary supplements using phenylketonuria as an example. *Mol Genet Metab.* 2012 Sep;107(1-2):3-9

Green Tea Extract Expert Panel

USP recently formed a Green Tea Extract Hepatotoxicity Expert Panel (USP GTEH EP) to review recent literature reports on green tea extract related hepatotoxicity. The GTEH EP will determine whether the cases of reported hepatotoxicity associated with green tea extracts are due to intrinsic factors of the green tea extract (toxicokinetics or others) or due to contaminants. The EP will also perform a health risk assessment on green tea extract to establish the levels of intake that warrant a cautionary labeling statement, if any. The EP expects to publish its findings in a peer-reviewed journal or other appropriate venue.

Public information on the safety of green tea extract currently under review by the USP Green Tea Extract Hepatotoxicity Expert Panel is welcome. For more information, please contact Hellen Oketch-R, Ph.D, at hao@usp.org.

Quality Leadership

Roundtable on USP Dietary Supplements Up-to-Date Standards October 25, 2016

Stakeholders from academia, regulatory agencies, ingredient manufacturers, finished product manufacturers, and trade associations participated in the discussion of how USP standards for dietary supplements need to be up to date, using current analytical procedures,

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Reference Standards in Development

Botanicals

- Cinnamaldehyde
- *Cinnamomum cassia* Twig Powder
- Oleonic Acid
- *Citrus reticulata* Pericarp Dry Extract
- Coffee Fruit Dry Extract
- Coix Seed Oil Extract
- D-Salicin
- Gallic Acid
- *Ligusticum chuanxiong* Rhizome Powder
- Nobiletin
- Red Clover Aerial Parts Isoflavone Aglycones Dry Extract
- *Salix* Species Bark Dry Extract
- Triolein
- Z-Ligustilide

Non-Botanicals

- Plant Stanol Esters
- Epicoprostanol
- Annatto Seed Tocotrienols Extract
- Citicoline Sodium
- Cobamamide
- Chia Seed Oil

and at the same time be affordable and relevant. The discussion topics at the meeting were (1) Prioritization of Monographs and Chapters in Need of Updating, (2) Up-to-Date Practices for USP Standards, (3) Up-to-Date Process and Communication, (4) Modern/Emerging Technologies and Tools, and (5) Planning the Compendial Future for Dietary Supplements.

Below is a summary of the feedback stakeholders provided at the roundtable:

- Prioritization criteria could include a risk of ingredient adulteration that leads to a public health issue, its prevalence in the market, and the level of consumption of it in products (i.e., how much it is used).
- Consider factors such as market share, relevance, and convenience.
- Omit monographs and general chapters that are no longer needed.
- Properly flag methods that lack specificity, so that potential for adulteration or contamination is understood.
- Omit methods that are outdated and no longer used (e.g., GC packed columns).
- Some companies cannot afford new technologies. It is important to retain equivalent old technologies while introducing new ones.
- Replace wet chemistry methods with instrumental methods. Change Thin-Layer Chromatography (TLC) to High-Performance Thin-Layer Chromatography (HPTLC). Change HPLC to UHPLC; NMR can be used for identification and quantitation. Inductively Coupled Plasma (ICP) to analyze metals is practically a universally established technique despite the considerable expense and running costs. Change to greener methods; for example, the use of supercritical mobile phases in chromatography will permit to cut on hazardous solvent use and toxic waste disposal.
- Send automatic electronic notification of relevant *USP–NF* content changes to customers.
- Provide an overview of USP's intentions to modernize monographs. This would allow companies to move forward using faster methods; those who cannot afford new methods could still use the older methods.

The following are other topics for which USP received feedback from participants:

- Adding HPTLC where TLC is missing; Adopting a new format allowing a comparison of multiple samples in the Dietary Supplements and Herbal Medicines Compendia; collaboratively validating several methods, and creating an electronic HPTLC image database
- Challenges to incorporating chemometrics
- New technologies to detect adulteration
- Use of a non-targeted method to identify outliers such as HPTLC, Quadrupole Time of Flight (QTOF), HPLC-MS
- Planning the compendial future for dietary supplements:
 - Share data packages and the analytical information behind monographs.

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[Find a Reference Standard](#)

[Suggest a Reference Standard](#)

[Purchase 2015 USP Dietary Supplements Compendium](#)

Monographs

Monographs in Revision Bulletin

- L-5-Methyltetrahydrofolate Tablets
(posted September 25, 2016; official November 1, 2016)
- L-5-Methyltetrahydrofolate Capsules
(posted September 25, 2016; official November 1, 2016)

Standards Open for Public Comment Until December 31, 2016

The standards below were published for public comment on September 1, 2016, and will receive and consider feedback until December 31, 2016. To comment, please visit: <http://www.usp.org/usp-nf/pharmacopeial-forum>.

New Monographs

- Tangerine Peel
- Tangerine Peel Dry Extract
- Tangerine Peel Powder

- Provide rationale for including and excluding parts of methods.
- Include validation data with a method so that a company knows how much it needs to validate.
- Consider upgrading units of measurement in monographs.
- Consider reducing the cost of Reference Standards to discourage use of secondary standards.
- Companies are developing their own methods to fit specific products. USP should be the driving force to help harmonize the methods.
- Seek existing methods adopted by other organizations and trade associations.

Research and Innovation

Seven New USP Admission Evaluations Move Ahead to Monograph Development

Prior to monograph development, dietary ingredients proposed for dietary supplement monograph development must undergo USP Admission Evaluation according to the guidelines available at http://www.usp.org/sites/default/files/usp_pdf/EN/dietarySup/admission_guideline_vers_1.1.pdf.

The admission evaluation process determines whether a dietary ingredient qualifies for admission into the *USP–NF* monograph development process based on its level of safety concern.

The evaluation process is done by the USP Dietary Supplements Admission Evaluations Joint Standard Setting Subcommittee (DSAE JS3). The DSAE JS3 reviews information related to safety, presence in the market, regulatory status, presence in the other pharmacopeias, etc., and determines whether the article poses a safety concern. If the article does not pose a safety concern, or poses a minor safety concern that can be mitigated by a label caution statement in the monograph, the article is placed in Class A, meaning that it is admitted for monograph development. If the article poses a safety concern it is placed in Class B and is not admitted for monograph development.

The USP DSAE JS3 reviewed the safety information for *Lactobacillus acidophilus* NCFM, *Lactobacillus acidophilus* LA-14, *Lactobacillus rhamnosus* HN001, *Bifidobacterium* Species, *Cinnamomum cassia* Twig, *Rhodiola crenulata*, Chia Seed Oil, and all seven articles were categorized into Class A, thus admitted for *USP–NF* monograph development according to the admission guideline.

Admission evaluations are in progress for the following nine articles: Red Clover Isoflavone Aglycones Extract, Coffee Fruit Dry Extract, Annatto Seed Tocotrienols, Cobamamide, Conjugated Linoleic Acid-Free Fatty Acids, Conjugated Linoleic Acid-Triglycerides, Tangerine Peel, Citicoline Sodium, and Calcium Citrate Malate.

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Monographs Open for Public Comment Until January 31, 2017

The monographs below were published for public comment on November 1, 2016, and will receive and consider feedback until January 31, 2017. To comment, please visit: <http://www.usp.org/usp-nf/pharmacopeial-forum>.

- Citicoline Sodium
- Coix Seed
- Coix Seed Powder
- Echinacea Species Powder Capsules

New Monographs Under Development

Botanicals

- *Bacillus coagulans* 5856
- Cranberry Juice-Derived Powder
- Dong Quai Root
- Dong Quai Root Powder
- Elderberry Dry Extract
- Eleuthero Root and Rhizome Powder Capsules
- Eleuthero Root and Rhizome Powder Tablets
- Ginkgo Capsules/Tablets
- Red Clover Tablets
- *Saccharomyces boulardii*
- Sichuan Lovage Rhizome
- Sichuan Lovage Rhizome Powder

Public information on safety of the articles under consideration by the DSAE JS3 for admission into the *USP–NF* is welcome. For more information, please contact Hellen Oketch-R, Ph.D, at hao@usp.org.

Nomenclature of Dietary Supplements

Updates From the Dietary Supplements and Herbal Medicines (DSHM) Nomenclature Subcommittee

From July 1 to September 30, 2016, the 2015–2020 Dietary Supplements and Herbal Medicines Nomenclature Joint Subcommittee (DSHM Nom JS) completed two important tasks. The subcommittee decided to recommend the name “Chewable Gels” for dosage forms commonly in the dietary supplement industry known as “gummies.”

The Nomenclature and Labelling Expert Committee (NL EC) adopted the name “Chewable Gels” by a unanimous vote. A revision to the definition of “Gels” in General Chapter <1151> *Pharmaceutical Dosage Forms* is requested to include current uses of these types of gels that conserve their shape as vehicles to deliver discrete dosage units of the active ingredients. Three monograph titles for chewable gels have since been approved by the NL EC.

The subcommittee finalized the Dietary Supplements Nomenclature Guideline, which has been forwarded to the NL EC for endorsement. The guideline is expected to be posted on the USP website by Winter 2016.

The following monographs approved by the Expert Committee:

- Carotenes
- Ascorbic Acid Chewable Gels
- Cholecalciferol Chewable Gels
- Oil- and Water-Soluble Vitamins with Minerals Chewable Gels
- Norway Spruce Lignans
- L-alpha-Glycerolphosphorylcholine
- Dong Quai Root
- Dong Quai Root Powder
- *Bacillus coagulans* GBI-30, 6086 Capsules
- Probiotics (GC)

Non-Botanicals

- Calcium Magnesium Citrate
- L-alpha-Glycerolphosphorylcholine
- Menaquinone-4
- Omega-3 Free Fatty Acids

The following monographs changed to conform to the Dietary Supplements Nomenclature Guideline

- Horse Chestnut changed to Horse Chestnut Seed
- Powdered Horse Chestnut changed to Horse Chestnut Seed Powder
- Powdered Horse Chestnut Extract changed to Horse Chestnut Seed Dry Extract
- Powdered Ashwagandha Root changed to Ashwagandha Root Powder
- Powdered Ashwagandha Root Extract changed to Ashwagandha Root Dry Extract

For more information on nomenclature issues, please contact Hellen Oketch-R, Ph.D, at hao@usp.org.

USP Answers Your Questions

Q. What are nominal concentrations and how are they applied in the calculation formulas?

A. Where a “nominal concentration” is specified as a term in a formula, such value is calculated as the concentration of the substance based on the label claim. For example, in the Assay for Niacin tablets, the nominal concentration is applied as follows.

Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) in the portion of tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Niacin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of niacin in the *Sample solution* (mg/mL)

Q. How and when do I need to correct my results for water content?

A. In assay procedures, water correction is typically stated in the definition and on the label of the USP Reference Standard. For other procedures, correction for assayed content, potency, or both is made prior to using the concentration in the equation provided in the monograph.

Q. How are the elemental impurities controlled in dietary supplements?

A. Effective January 1, 2018, elemental contaminants will be controlled in official dietary supplements according to the principles defined and requirements specified in *Elemental Contaminants in Dietary Supplements* <2232>.

Also effective January 1, 2018, *Heavy Metals* <231> will be omitted and all references to it in general chapters and monographs will be deleted. Early adoption of the requirements in <2232> is permitted by USP, and <233>, as applicable, is fully implemented with respect to a particular dietary supplement in advance of the January 1, 2018, date. Products and ingredients that comply with the requirements in <2232> in advance of the January 1, 2018 date will be considered to be in conformance with *USP–NF* requirements.