AOAC Stakeholder Panel on Strategic Food Analytical Methods: Stakeholder Panel Meeting

Meeting Minutes
March 17, 2016; 8:30 a.m. – 4:30 p.m. ET

Attendees

Stakeholder Panel Members (Present during all or part of the meeting):

Karen Andrews, USDA
John Austad, Covance
Joseph Betz, NIH
Joe Boison, CFIA
Carolyn Burdette, NIST
Spencer Carter, Genysis Labs
Bob Clifford, Shimadzu
Jason Cooley, Biocell Technology
Tony Crosaroil, Eurofins
Steven Dentali, Herbalife
Linda Dodd, PB Leiner
Christine Farthing, Pfizer
Heather Figure, Healthy Directions
Nicole Hart, Agilent Technologies
Jana Hildreth, Synutra Pure
Norma Hill, TTB (retired), AOAC President
Mark Hokenson, Pharmavite
Suhail Ishaq, Biocell Technology
Martha Jennens, Covance
Holly Johnson, Alkemist Labs
George Joseph, AsureQuality
Mohamed Koroma, Pharmavite
Adam Kuszak, NIH
Tom Lawson, VitaQuest
Sookwang Lee, FDA
Andy Lipper, Weber State
Elaine Marley, R-Biopharm

Mary McBride, Agilent
Paul Milne, Keurig Green Mountain
Elizabeth Mudge, BCIT
Maria Ofitserova, Pickering Labs
Punam Patel, Pharmavite
Melissa Phillips, NIST
Curtis Phinney, Curtis Phinney CNS
Gunther Raffler, Eurofins
Kate Rimmer, NIST
Shauna Roman, RB
Leila Saldanah, NIH
Myron Sasser, MIDI
Brooke Schwartz, Brooke Schwartz Consulting
Aniko Solyom, GAAS Analytical
William Sommer, Natto Pharma
John Szpylka, Mérieux NutriSciences
John Travis, NSF International
Socrates Trujillo, FDA
Denise Walters, Pfizer
Michael Weipert, Living Essentials
Laura Wood, NIST
Jason Wubben, ADM
William Xue, Accucaps LTD
Jincai Yang, NBty Inc.
Dorothy Young, Agilent
Kurt Young, GNC
Weiguo Zhang, Synutra Pure
Joseph Zhou, Sunshineville Health Products
Joyce Zhu, Jamieson Lab
Garrett Zielinski, Covance
Jerry Zweigenbaum, Agilent

AOAC Staff (Present during all or part of the meeting):

Jim Bradford
Scott Coates
Christopher Dent
Jonathan Goodwin
Arlene Fox
Dawn Frazier

Nora Marshall
Deborah McKenzie
Tien Milor
La’Kia Phillips
Joyce Schumacher
Robert Rathbone
Meeting Minutes

I. **Welcome and Introductions**

Bradford opened the meeting at approximately 8:30 a.m. ET and introduced AOAC President Norma Hill, who provided opening remarks. SPDS Chair Darryl Sullivan then took the floor and reviewed the day’s agenda.

II. **Project Overview and Updates**

Sullivan took the floor and highlighted the AOAC policies and procedures found in the meeting eBook. He then proceeded with a presentation\(^1\) on the project in general and progress on previous ingredients and noted that the Call for Methods and Call for Experts for Aloin, Cinnamon, Tea, and Vitamin D will be live on the AOAC website until Friday, April 29.

III. **SMPR Presentations**

**Collagen**

Cooley took the floor with a presentation\(^2\) on the Collagen SMPR. Cooley reviewed the fitness for purpose, the background on collagen, and the members and activities of the working group, the various types of collagen, and the key points of the draft SMPR. He then opened the floor for discussion. A participant asked a question about the applicability, “The method will be able to identify and quantify individual native (un-denatured) and hydrolyzed collagen type I, II & III if one or multiple types are present in dietary ingredients and dietary supplement finished products.” Are there methods that can do all of this? Cooley replied that it may be a challenge but the working group discussed this and agreed that methods are needed for all of this.

He then made a motion to accept the Collagen SMPR as presented.\(^3\)

**MOTION by Cooley / Ishaq to approve the SMPR for Quantitation of Collagen as presented. 16 in favor, 0 opposed, 1 abstained. The motion passed.**

**Lutein**

Sullivan advised that the Chair of the Lutein Working Group was unable to attend this meeting and AOAC Chief Science Officer Scott Coates will present slides\(^4\) on his behalf. Coates reviewed the fitness for purpose, the background on lutein, and the members and activities of the working group, the various types of lutein, the comments submitted, and the key points of the draft SMPR. Coates then asked for a motion to approve the document.

**MOTION by Barrett / Solyom to approve the SMPR for Quantitative measurement of β-cryptoxanthin, lutein, and zeaxanthin in ingredients and dietary supplements as presented.**

\(^1\) Sullivan Presentation  
\(^2\) Cooley Presentation  
\(^3\) Collagen SMPR As approved  
\(^4\) Myers Presentation
Discussion followed this motion. The group agreed to change the applicability to specify cis and trans isomers and also added “and their fatty acid esters”. The group also added the words “matrix and analyte in total” to the footnote in Table 2.

Barrett and Solyom accepted the modified motion to accept the SMPR as amended\(^5\) and Sullivan called the question.

**16 in favor, 0 opposed, 1 abstained. The motion passed.**

**Turmeric**

Sullivan introduced Solyom, who took the floor with a presentation\(^6\) on the Turmeric Working Group. Solyom reviewed the fitness for purpose, the background on turmeric, and the members and activities of the working group, the comments submitted, and the key points of the draft SMPR. She then opened the floor for discussion. A number of stakeholders were concerned about the range presented in the SMPR. After some discussion, the group agreed to modify the ranges for recovery, % RSD\(_r\) and % RSD\(_R\). These changes were made to the SMPR\(^7\) in real time.

**MOTION by Solyom/Andrews to approve the SMPR for Quantitation of Curcuminoids as amended. 16 in favor, 0 opposed, 1 abstained. The motion passed.**

**IV. Working Group Launch Presentations**

**Aloe Vera Working Group Launch**

Sullivan invited Kan He to the floor as the Chair for the working group on Aloe Vera. Kan He provided a presentation\(^8\) to launch the new working group. He reviewed the background of aloe vera, its significance, existing methods, and the analytical needs. He concluded with a proposed fitness for purpose:

“The methods are able to qualitatively identify aloe vera; are able to accurately quantitate not only the contents of aloe polysaccharides, but also the molecular weight; are able to accurately quantitate the aloe polysaccharides with different molecular weight.”

With the presentation concluded, He opened the floor to questions and comments. The stakeholders agreed that this will be a challenging topic that will result in multiple SMPRs. By straw poll, the voting members agreed to the proposed fitness for purpose and the Aloe Vera Working Group was formally launched.

**Protein Working Group Launch**

Sullivan invited Carter to the floor as the Chair of the working group for Protein. Carter provided a presentation\(^9\) regarding the background of aloe vera, its significance, challenges, and the analytical needs. He concluded with the following fitness for purpose proposal:

---
\(^5\) Lutein SMPR as approved  
\(^6\) Solyom Presentation  
\(^7\) Turmeric SMPR as approved  
\(^8\) He Presentation  
\(^9\) Carter Presentation
“Qualitative method must provide positive confirmation of specific protein. Quantitative method must provide accurate and precise concentrations of specific proteins in raw materials and finished goods. Orthogonal methods may be required due to complex approach to analysis.”

Carter then opened the floor for discussion. There was a question about hydrolyzed proteins and these were determined to be out of scope. The group also determined that this will likely require two (2) SMPRs. After further discussion the fitness for purpose was refined to read:

“Method must identify and quantify specific proteins in presence of other proteins and potential adulterants. Quantitative method must provide accurate and precise concentrations of specific intact proteins in ingredients and finished goods.”

By straw poll, the voting members agreed to the proposed fitness for purpose and the Protein Working Group was formally launched.

Vitamin B12 Working Group Launch

Sullivan invited van Breemen to the floor as Chair of the Vitamin B12 Working Group. Van Breemen provided a presentation\(^\text{10}\) on the background, analytical challenges, regulatory issues, and existing methods for Vitamin B12. He concluded with the following fitness for purpose proposal:

“The method for vitamin B\(_{12}\) dietary supplement analysis must quantitate multiple forms of vitamin B\(_{12}\) individually or after conversion to a common form (such as the more stable cyanocobalamin) in a variety of dosage forms. The method must also be able to distinguish between active vitamin B\(_{12}\) corrinoids and inactive forms present in products derived from some microbiological sources. As humans can only absorb 10 to 500 μg B\(_{12}\)/day and the RDA is from 0.4 to 2.8 μg B\(_{12}\)/day, the analytical range for supplements should extend from at least 0.1 to 1000 ppm per dosage unit.”

After a group discussion, the statement was modified to read:

“The method for vitamin B\(_{12}\) dietary supplement analysis must quantitate multiple forms of vitamin B\(_{12}\) individually in a variety of dosage forms. The method must also be able to distinguish between active vitamin B\(_{12}\) corrinoids and inactive forms present in products derived from some microbiological sources.”

By straw poll, the voting members agreed to the proposed fitness for purpose and the B\(_{12}\) Working Group was formally launched.

V. Next Steps and Adjourn

Sullivan thanked the presenters and all present for their participation. He advised the group that the working groups are open to all and to sign up for participation at using the form\(^\text{11}\) provided in the meeting book. He also reiterated that the Call for Methods and Call for Experts for Aloin, Cinnamon, Tea, and Vitamin D will be live on the AOAC website until Friday, April 29; and the ERP for these ingredients is planned for June, 2016. The meeting adjourned at approximately 4:30 p.m. ET.

Action Items

\(^{10}\) Van Breemen Presentation

\(^{11}\) https://form.jotform.com/60285694384163
1. Add definition of “total lutein” to approved Lutein SMPR

Attachments

1. Sullivan Presentation
2. Cooley Presentation
3. Collagen SMPR As approved
4. Myers Presentation
5. Lutein SMPR as approved
6. Solyom Presentation
7. Turmeric SMPR as approved
8. He Presentation
9. Carter Presentation
10. Van Breemen Presentation
Update on the Stakeholder Panel on Dietary Supplements (SPDS)

Darryl Sullivan, Chair
Stakeholder Panel on Dietary Supplements
Covance Laboratories

March 2016

AOAC SPDS History

- AOAC INTERNATIONAL signed a 5-year contract with the National Institutes of Health-Office of Dietary Supplements (NIH/ODS) to establish voluntary consensus standards for high-priority ingredients.

- Develop 25 standard method performance requirements (SMPRs) for priority dietary supplement ingredients.

- Deliver First Action Official MethodsSM for the prioritized dietary supplement ingredients.

- Encourage participation with the dietary supplements industry to develop voluntary consensus standards.
AOAC SPDS 5 Year Plan

• **5 Advisory Panel Meetings** to identify key stakeholders, subject matter experts, frames the issues, determine ingredients, and set priorities for the stakeholder panel.

• **10 Stakeholder Panel Meetings** to deliberate and approve voluntary consensus standards.

• **25 Total Working Groups** to draft and recommend SMPRs.

• **8 Expert Review Panel Meetings** to review and potentially adopt fit for purpose First Action Official Methods℠ for 25 ingredients.

Stakeholder Panel on Dietary Supplements (SPDS)

• **Update on Ingredients:**
  — Set 2 ERP held on December 2015
    • Ashwagandha, Folin C, and Mitragyna speciosa – 1 AOAC First Action Official Method℠ Status for Ashwagandha
    • Cinnamon ERP slated for June 2016
  — Set 3 Call for Methods and Experts posted on AOAC Web site
    • Aloe, Tea, and Vitamin D
    • ERP slated for June 2016
  — Set 4 SMPRs to be recommended at AOAC SPDS March 2016
    • Collagen, Lutein (and Esters), and Turmeric
### Stakeholder Panel on Dietary Supplements (SPDS)

<table>
<thead>
<tr>
<th>Update on Ingredients:</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Launch for set 5 slated for 2016 AOAC Midyear SPDS Meeting</td>
</tr>
<tr>
<td>- Aloe vera, Chair - Kan He (Herbalife)</td>
</tr>
<tr>
<td>- Protein, Chair - Spencer Carter (Genysis Labs)</td>
</tr>
<tr>
<td>- Vitamin B₁₂, Chair - Richard van Breeman (University of Illinois at Chicago)</td>
</tr>
<tr>
<td>- Launch for set 6 slated for 2016 AOAC Annual Meeting SPDS Meeting</td>
</tr>
<tr>
<td>- Vitamin K₁ and K₂, Chair TBD</td>
</tr>
<tr>
<td>- Free amino acids, Chair TBD</td>
</tr>
<tr>
<td>- Ginger, Chair TBD</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SPDS Advisory Panel slated for fall 2016 to prioritize next 6 ingredients for 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Advisory Panel includes representatives from AHPA, CRN, CHPA, NSF, NPA, NIH, USP, Herbalife, and Synutra Pure</td>
</tr>
</tbody>
</table>
AOAC SPDS Publications

• Nutraceuticals World
  – Six More Dietary Ingredients Picked for Analytical Evaluation, by Richard A. Lovett, JD, PhD

• JAOAC
  – Jan/Feb Articles on Chondroitin and PDE5 Inhibitors
  – Encourage submit work to JAOAC

Call for Methods and Call for Experts

• Call for Method and the Call for Experts is posted on the AOAC web site for the set 3 ingredients:
  – Aloin in Aloe
  – Tea
  – Vitamin D

• Deadline for Methods and CVs is April 29
**How do you get involved?**

- Submit methods on the Call for Methods tab at [www.aoac.org](http://www.aoac.org)
- Volunteer for Expert Review Panels on the Call for Experts tab at [www.aoac.org](http://www.aoac.org)
- SPDS site at [www.aoac.org](http://www.aoac.org), click “Standards”, then Stakeholder Panel on Dietary Supplements (SPDS) for complete information about the program

**Contact Information**

Darryl Sullivan, Chair SPDS  
*Covance Laboratories*  
Tel: 608.242.2711  
Email: darryl.sullivan@covance.com

Contact AOAC Staff:  
Tel: 301.924.7077  
Web: [www.aoac.org](http://www.aoac.org)  
- **Jim Bradford**, Executive Director/CEO, jbradford@aoac.org, ext. 102  
- **Deborah McKenzie**, Sr. Director, Standards Development and AOAC Research Institute, dmckenzie@aoac.org, ext. 157  
- **Dawn Frazier**, Sr. Executive for Scientific Business Development, dfrazier@aoac.org, ext. 117
AOAC INTERNATIONAL
STAKEHOLDER PANEL ON
DIETARY SUPPLEMENTS
Suhail Ishaq and Jason Cooley, BioCell
Collagen Working Group
March 17, 2016
Gaithersburg, Maryland

Fitness for Purpose

The method should be able to:

Quantify total native (undenatured) and hydrolyzed collagen type I, II & III in the raw materials and final finished dosage forms including but not limited to dry powders, tablets, capsules, softgels and liquids. Individually separate and quantify native (undenatured) and hydrolyzed collagen type I, II & III if blended together.
Collagen Working Group Members

- Suhail Ishaq, BioCell Technology (Chair)
- Ali Asim, BioCell Technology
- Maria Bojstrup, Pfizer
- Jason Cooley, BioCell Technology
- Linda Dodd, PB Gelatins/PB Leiner
- Christine Farthing, Pfizer
- Prashang Ingle, Herbalife
- Adam Kuszak, NIH
- Elizabeth Mudge, BCIT
- Curtis Phinne, Curtis Phinne CNS
- Lars Reimann, Eurofins
- Brian Schaneberg, Starbucks
- Darryl Sullivan, Covance
- John Szpylka, Merieux Nutrisciences
- John Travis, NSF International
- Denise Walters, Pfizer
- Kurt Young, GNC/Nutra Manufacturing
- Joseph Zhou, Sunshineville Health Products
- Garrett Zielinski, Covance

Collagen Working Group
Work to Date

• 1 In Person Meeting

• 3 teleconferences (November 2015 – December 2015)

• 1 SMPR Drafted

• Public comment period (January 8, 2016 – February 5, 2016)

• SMPRs made ready for SPDS review and approval
Background

Collagen:

- Main structural protein in the extracellular space in various connective tissues in animals.
- Primary component of connective tissue
- Most abundant protein in mammals (~25% to 35% of protein content).
- Over 30 “Types”:
  1. Fibrillar (Types I, II, III, V, XI)
  2. non-fibrillar (all the rest).

Background

Collagen structure:

Connects/supports organs & tissues
(e. g. skin, bone, blood vessels, tendons, muscles, and cartilage)
Background

Collagen structure (hydroxyproline):  
Wealth of hydroxyproline is marker of collagen fibrils

Main Collagen Types

<table>
<thead>
<tr>
<th>Type</th>
<th>Location/Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Skin, Tendons, Bones, Arteries, Cornea, Scar tissue</td>
</tr>
<tr>
<td>II</td>
<td>Joints, Hyaline cartilage, vitreous humour</td>
</tr>
<tr>
<td>III</td>
<td>Skin, granulation tissue, reticular fiber</td>
</tr>
<tr>
<td>IV</td>
<td>Basal lamina, eye lens, capillaries, kidney</td>
</tr>
</tbody>
</table>

More than 14 types defined, but types I-IV are most abundant
Amino acid sequences differ between collagen proteins by > 40% sequence identity

Posttranslational modifications are different as well.

E.g., type II α1 protein can have ten-fold more hydroxylation at Proline and glycosylation events at its lysine residues than similar Type I protein.
Commercial Collagen Products

1. Gelatin:
   • an irreversibly denatured (Heat or Acid) form of collagen (usually types I & III) used in food and cosmetics industry

2. Partially denatured (physical breakdown) or non-hydrolyzed:
   • All types (I/III and II are most common)
   • High molecular weight
   • Limited water solubility (soluble in mildly acidic solutions)

3. Hydrolyzed
   • Type I/III (derived from beef, pig or fish skin and bones)
   • Type II (usually from chicken sternal cartilage), can be

SMPR Key Points

Applicability:
The method will be able to identify and quantify individual native (undeveloped) and hydrolyzed collagen type I, II & III if one or multiple types are present in dietary ingredients and dietary supplement finished products.

Validation Guidance:
Data demonstrating that a candidate method is able to: Separate a combination of native collagen type I, II and III and/or hydrolyzed collagen type I, II and III. Quantify each individual collagen type both native and hydrolyzed.

Table 1: Method performance requirements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical Range (%)</td>
<td>1 – 100</td>
</tr>
<tr>
<td>LOQ (%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>90-110</td>
</tr>
<tr>
<td>% RSD_r</td>
<td>≤ 5</td>
</tr>
<tr>
<td>% RSD_R</td>
<td>≤ 10</td>
</tr>
</tbody>
</table>
Comments Submitted (if any)

- No comments submitted

Motion

- Move to accept the Standard Method Performance Requirements for Collagen as presented.
Quantitation of Collagen

Intended Use: Reference method for cGMP compliance.

1. Purpose: AOAC SMPRs describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are written and adopted by AOAC Stakeholder Panels composed of representatives from the industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC Expert Review Panels in their evaluation of validation study data for method being considered for Performance Tested Methods or AOAC Official Methods of Analysis, and can be used as acceptance criteria for verification at user laboratories.

2. Applicability:
The method will be able to identify and quantify individual native (un-denatured) and hydrolyzed collagen type I, II & III if one or multiple types are present in dietary ingredients and dietary supplement finished products.

3. Analytical Technique:
Any analytical technique(s) that measures the analytes of interest and meets the following method performance requirements is/are acceptable.

4. Definitions:

Collagen
A triple helix protein that generally consists of two identical chains (α1) and an additional chain that differs slightly in its chemical composition (α2). The amino acid composition of collagen is notable for its particularly high hydroxyproline content. The three most common types of collagen are: type I, found in skin, tendon, vascular ligature, organs, bone (main component of the organic part of bone); type II, found in cartilage (main collagenous component of cartilage); and type III, found in reticular fibers.

Structures:

Dietary Ingredients
A vitamin; a mineral; an herb or other botanical; an amino acid; a dietary substance for use by man to supplement the diet by increasing total dietary intake; or a concentrate, metabolite, constituent, extract, or combination of any of the above dietary ingredients.¹

Dietary supplements

¹ Federal Food Drug and Cosmetic Act §201(ff) [U.S.C. 321 (ff)
A product intended for ingestion that contains a "dietary ingredient" intended to add further nutritional value to (supplement) the diet. Dietary supplements may be found in many forms such as tablets, capsules, softgels, gelcaps, liquids, or powders.

**Hydrolyzed Collagen**

Peptides and polypeptides rich in hydroxyproline, produced by breaking down the molecular bonds of native collagen strands using one or more combinations of physical, chemical, or biological methods.

**Limit of Quantitation (LOQ)**

The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

**Quantitative method**

Method of analysis whose response is the amount of the analyte measured either directly (enumeration in a mass or a volume), or indirectly (color, absorbance, impedance, etc.) in a certain amount of sample.

**Repeatability**

Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator and repeating during a short time period. Expressed as the repeatability standard deviation (SD_r); or % repeatability relative standard deviation (%RSD_r).

**Reproducibility**

The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD_R); or % reproducibility relative standard deviation (% RSD_R).

**Recovery**

The fraction or percentage of spiked analyte that is recovered when the test sample is analyzed using the entire method.

5. **Method Performance Requirements:**

See table 1.

6. **System suitability tests and/or analytical quality control:**

Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range.

7. **Reference Material(s):**


Identify suitable materials for method validation

8. **Validation Guidance:**

Requirement for consideration as an AOAC Official Methods of Analysis:
Data demonstrating that a candidate method is able to: Separate a combination of native collagen type I, II and III and/or hydrolyzed collagen type I, II and III. Quantify each individual collagen type both native and hydrolyzed.


9. Maximum Time-To-Result: None
Table 1: Method performance requirements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical Range (%)</td>
<td>1 – 100</td>
</tr>
<tr>
<td>LOQ (%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>90-110</td>
</tr>
<tr>
<td>% RSDr</td>
<td>≤ 5</td>
</tr>
<tr>
<td>% RSDR</td>
<td>≤ 10</td>
</tr>
</tbody>
</table>

Table 2: Matrices

- tablets
- capsules
- softgels
- powders
- liquids
- chewables
Fitness for Purpose

Quantitative measurement of the following in both raw materials and dietary supplements:

- Lutein
- 3’-Epilutein
- Zeaxanthin
- β-Cryptoxanthin
### Lutein Working Group Members

<table>
<thead>
<tr>
<th>Member</th>
<th>Organization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rick Myers, Kemin (Chair)</td>
<td>Lanette Richards, TBAR</td>
</tr>
<tr>
<td>Maria Bøjstrup, Pfizer</td>
<td>Catherine Rimmer, NIST</td>
</tr>
<tr>
<td>Neil Craft, Craft Technologies</td>
<td>Brian Scheneberg, Starbucks</td>
</tr>
<tr>
<td>April Hall, Nutra Manufacturing</td>
<td>Aniko Solyom, GAAS Analytical</td>
</tr>
<tr>
<td>Fred Khachik, Kemin Industries</td>
<td>Darryl Sullivan, Covance</td>
</tr>
<tr>
<td>David Kennedy, Phenomenex</td>
<td>John Spzylka, Mérieux</td>
</tr>
<tr>
<td>Elizabeth Mudge, BCIT</td>
<td>NutriScience</td>
</tr>
<tr>
<td>Melissa Phillips, NIST</td>
<td>Denise Walters, Pfizer</td>
</tr>
<tr>
<td>Tom Phillips, MD Department of Agriculture</td>
<td>Jinchaun Yang, Waters</td>
</tr>
<tr>
<td></td>
<td>Tyler White, TBAR</td>
</tr>
<tr>
<td></td>
<td>Garrett Zielinski, Covance</td>
</tr>
</tbody>
</table>

### Lutein Working Group

#### Work to Date

- 1 in-person meeting
- 3 teleconferences (November 2015 – December 2015)
- 1 SMPR Drafted
- Public comment period (January 8, 2016 – February 5, 2016)
- SMPRs made ready for SPDS review and approval
Background

Carotenoids are a diverse family of botanical pigments
Minimal biosynthesis in animals; so must derive from diet

Botanical function
  Mediate photoinduced electron transfer to chlorophyll
  Quench singlet/triplet-chlorophyll that can damage allied tissues during very active photosynthesis

Two relevant carotenoid families
  Carotenes: hydrocarbons (orange)
  Xanthophylls: hydroxylated carotenes (yellow)

Only xanthophylls of interest here. Dozens exist!
• Often exists as fatty acid esters in nascent tissue

1. Lutein

  o (3R,3’R,6’R)-β,ε-Carotene-3,3’-diol; dietary
  o Commercial and supplemental roles
    • Accumulates throughout human retina
    • Reportedly rescues AMD
    • Present in other tissues, relevance under study
    • Colors white egg yolks yellow
    • Antioxidant
    • Colorant (E161b)
  o Structure

  o Proposed daily dose: 10 mg
2. **Zeaxanthin**

- $\beta,\beta'$-Carotene-3,3'-diol; *dietary*
- Zeaxanthin differs from lutein only by placement of single double bond.
- Commercial and supplemental roles
  - Also accumulates in human retina; predominates in *macula lutea*
  - Reportedly rescues AMD
  - Colors white egg yolks yellow
  - Colorant (E161h)
- Structure
- Proposed daily dose: 2 mg

3. **$\beta$-Cryptoxanthin**

- (3R,3'R,6'R)-$\beta,\varepsilon'$-Carotene-3,3'-diol; *dietary*
- Commercial and supplemental roles
  - Provitamin in humans; converted to vitamin A
  - Possible antioxidative DNA protection, bone health, others
  - Colorant (E161c) in Australia and New Zealand; not in US or EU
- Structure
- Proposed daily dose: 4 mg
4. 3’-Epilutein

- (3R,3’S,6’R)-Lutein
- **Not dietary**—no biological or commercial role
- Significant epimer product and loss of lutein
  - Occurs in aqueous acid
  - Reaction likely proceeds by $S_N1$ and $S_N2$, but mostly $S_N2$ since conversion exceeds 50%
- Structure

General Analytical Needs

**Method should**

- Quantitatively de-esterify all analyte forms
- Separate and accurately quantify relevant free analytes
  - Lutein
  - Zeaxanthin
  - β-Cryptoxanthin
  - 3’-Epilutein (principal lutein metabolite)
- Determine the above in
  - Raw materials used in dietary supplement formulations
  - Finished products
SMPR criteria

Applicability

Separate quantitative determination of β-cryptoxanthin, lutein, and zeaxanthin in ingredients and dietary supplements.
Analytical Technique(s)

Any analytical technique that resolves and quantifies the analytes of interest and meets the following method performance requirements is acceptable.

Method Performance Requirements
# Analytical Range and LOQ Requirements

<table>
<thead>
<tr>
<th>Analytical Range</th>
<th>0.0005% to 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 to 1,000,000 ppm</td>
</tr>
<tr>
<td>Limit of Quantitation (LOQ)</td>
<td>2ppm</td>
</tr>
<tr>
<td></td>
<td>0.0002%</td>
</tr>
</tbody>
</table>

# Recovery, Repeatability, and Reproducibility Parameters

<table>
<thead>
<tr>
<th>Range</th>
<th>5 to 20 ppm</th>
<th>&gt;20 to 1000 ppm</th>
<th>&gt;0.1% to 1%</th>
<th>&gt;1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery</td>
<td>80 to 110%</td>
<td>95 to 105%</td>
<td>97 to 102%</td>
<td>98 – 102%</td>
</tr>
<tr>
<td>Repeatability</td>
<td>8</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>12</td>
<td>8</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>
Matrices

- Tablets
- Capsules
- Liquids
- Powders
- Extracts
- Plant products

Comments Submitted

1. Delete “NIST list of lutein, zeaxanthin, and β-cryptoxanthin in foods” since levels are much too low and not applicable. **DONE**
2. Change reference material entry to read “NIST SRM 3280 Multivitamin/Multi-element Tablets.” **DONE**
3. Typos: spelling (matrix, tables) and remove comma. **DONE**
Motion

• Motion to accept the Standard Method Performance Requirements for Lutein as presented.

Discussion?
SMPR Name: Quantitative measurement of \(\beta\)-cryptoxanthin, lutein, and zeaxanthin in ingredients and dietary supplements.

Intended Use: Reference method for cGMP compliance.

1. Purpose

AOAC SMPRs describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are written and adopted by AOAC Stakeholder Panels composed of representatives from the industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC Expert Review Panels in their evaluation of validation study data for method being considered for Performance Tested Methods or AOAC Official Methods of Analysis, and can be used as acceptance criteria for verification at user laboratories. [Refer to Appendix F: Guidelines for Standard Method Performance Requirements, Official Methods of Analysis of AOAC INTERNATIONAL (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA.]

2. Applicability:

Separate quantitative determination the cis and trans isomers of \(\beta\)-cryptoxanthin, lutein, and zeaxanthin and their fatty acid esters in ingredients and dietary supplements.

3. Analytical Technique:

Any analytical technique(s) that measures the analytes of interest and meets the following method performance requirements is/are acceptable.

4. Definitions:

Analytes

**\(\beta\)-Cryptoxanthin**

IUPAC name: (R)-3,5,5-Trimethyl-4-[3,7,12,16-tetramethyl-18-(2,6,6-trimethylcyclohex-1-enyl)-octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]-cyclohex-3-enol. CAS registry number: 472-70-8. See figure 1 for chemical structure.

**Lutein**

IUPAC name: \(\beta,\varepsilon\)-carotene-3,3'-dil. CAS registry number 1 27-40-2. See figure 2 for chemical structure.

**Zeaxanthin**

IUPAC name: 4-[18-(4-hydroxy-2,6,6-trimethyl-1-cyclohexenyl)-3,7,12,16-tetramethyl-octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]-3,5,5-trimethyl-cyclohex-3-en-1-ol. CAS registry number: 144-68-3. See figure 3 for chemical structure.
Dietary Ingredients
A vitamin; a mineral; an herb or other botanical; an amino acid; a dietary substance for use by man to supplement the diet by increasing total dietary intake; or a concentrate, metabolite, constituent, extract, or combination of any of the above dietary ingredients.¹

Dietary Supplements
A product intended for ingestion that contains a "dietary ingredient" intended to add further nutritional value to (supplement) the diet. Dietary supplements may be found in many forms such as tablets, capsules, softgels, gelcaps, liquids, or powders.

Limit of Quantitation (LOQ)
The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

Quantitative method
Method of analysis which response is the amount of the analyte measured either directly (enumeration in a mass or a volume), or indirectly (color, absorbance, impedance, etc.) in a certain amount of sample.

Repeatability
Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator and repeating during a short time period. Expressed as the repeatability standard deviation (SDr); or % repeatability relative standard deviation (%RSDr).

Reproducibility
The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility relative standard deviation (SDR); or % reproducibility relative standard deviation (% RSDR).

Recovery
The fraction or percentage of spiked analyte that is recovered when the test sample is analyzed using the entire method.

5. Method Performance Requirements:
See table 1 and 2.

6. System suitability tests and/or analytical quality control:
Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range.

7. Reference Material(s):

USP Lutein
USP Zeaxanthin

¹ Federal Food Drug and Cosmetic Act §201(ff) [U.S.C. 321 (ff)]
8. **Validation Guidance:**


All matrices in table 3 shall be evaluated, or the scope (applicability) of AOAC-adopted method must expressly state the applicable dietary supplement forms.

9. **Maximum Time-To-Result:** None
Table 1: Analytical Range and LOQ Requirements

<table>
<thead>
<tr>
<th>Analytical Range</th>
<th>0.0005% to 100%</th>
<th>5 to 1,000,000 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of Quantitation (LOQ)</td>
<td>≤ 0.0002%</td>
<td>≤ 2 ppm</td>
</tr>
</tbody>
</table>

Table 2: Recovery, Repeatability, and Reproducibility Parameters

<table>
<thead>
<tr>
<th>Range</th>
<th>5 to 20 ppm</th>
<th>&gt;20 to 1000 ppm</th>
<th>&gt;0.1% to 1%</th>
<th>&gt;1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Recovery</td>
<td>80 to 110</td>
<td>95 to 105</td>
<td>97 to 102</td>
<td>98 – 102</td>
</tr>
<tr>
<td>% RSDr</td>
<td>≤ 8</td>
<td>≤ 5</td>
<td>≤ 4</td>
<td>≤ 2</td>
</tr>
<tr>
<td>% RSDR</td>
<td>≤ 12</td>
<td>≤ 8</td>
<td>≤ 6</td>
<td>≤ 3</td>
</tr>
</tbody>
</table>

% recovery, % RSDr, and % RSDR shall be determined individually for each claimed matrix and analyte in total.

Table 3: Matrices

- Tablets
- Capsules
- Liquids
- Powders
- Extracts
- Plant products
- Gummies
Figure 1: Chemical structure of all-trans β-cryptoxanthin.

Figure 2: Chemical structure of all-trans lutein.

Figure 3: Chemical structure of all-trans zeaxanthin.
AOAC INTERNATIONAL
STAKEHOLDER PANEL ON
DIETARY SUPPLEMENTS

Anikó Sólyom, GAAS Analytical
Turmeric Working Group
March 17, 2016

Gaithersburg, Maryland
Fitness for Purpose

The method will be able to quantify total curcuminoid content, calculated as the sum of curcumin, demethoxycurcumin, and bis-demethoxycurcumin, in turmeric \textit{[Curcuma longa} Linn.] rhizome, powdered botanical raw materials, extracts, and dietary supplement finished products containing turmeric extract, alone or in combination with other dietary ingredients. The method must be able to separate and quantify each individual curcuminoid.
Anikó Sólyom, GAAS Analytical (Chair)
Joseph Betz, NIH ODS
Paula Brown, BCIT
Nicole Chrisafis, Gaia Herbs
David Kennedy, Phenomenex
Adam Kuszak, NIH ODS
Elizabeth Mudge, BCIT
Melissa Phillips, NIST
Tom Phillips, MD Department of Agriculture
Lanette Richards, TBAR
Kate Rimmer, NIST
Brian Schaneberg, Starbucks
Bernice Sauza, TBAR
Jules Skamarack, Eurofins
Darryl Sullivan, Covance
John Szpylka, Mérieux
NutriSciences
John Travis, NSF International
Jinchaun Yang, Waters
Joseph Zhou, Sunshineville
Turmeric Working Group
Work to Date

• 1 In Person Meeting

• 3 teleconferences (November 2015 – December 2015)

• 1 SMPR Drafted

• Public comment period (January 8, 2016 – February 5, 2016)

• SMPRs made ready for SPDS review and approval
Background

Turmeric (Curcuma longa L.)

Common names: turmeric, turmeric root, Indian saffron
Member of the ginger family, Zingiberaceae

Turmeric rhizoma
Background

Uses

Culinary:
• flavoring and coloring agent
• main spice in curry

Traditional Chinese and Ayurvedic medicine:
• topical application for eczema and wound healing
• aid digestion and liver function
• relieve arthritis pain
• regulate menstruation

Current research:
• osteoarthritis, Alzheimer disease, eye inflammation,
• colorectal cancer, Crohn’s disease, diabetes, stomach upset
• gingivitis, stomach ulcer, irritable bowel syndrome, RA and more.

Background

Turmeric (Curcuma longa L.)

Spectra of turmeric extract

Approx. 5% of the plant is curcumin
Background

Curcuminoids

Curcumin
MW:368

Demethoxycurcumin
MW:338

Bisdemethoxycurcumin
MW:308
In the Dietary Supplement Database (DSLD) 1,044 products contained turmeric and/or curcumin(oids) and/or extracts (out of total 42,000 DS)

47% of these products turmeric/carcumin as a component of a blend

Source: Leila G. Saldanha, PhD, RD, Office of Dietary Supplement, NIH. Personal communication
Background

Significance

190 clinical trials between 1996 and 2015
(http://clinicaltrials.gov)

<table>
<thead>
<tr>
<th>NCT Number</th>
<th>Recruitment</th>
<th>Conditions</th>
<th>Sponsor/Collaborators</th>
<th>Start Date</th>
<th>Phases</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02532023</td>
<td>Enrolling by invitation</td>
<td>Migraine</td>
<td>Tehran University of Medical Sciences</td>
<td>September 2015</td>
<td>Phase 4</td>
</tr>
<tr>
<td>NCT02532023</td>
<td>Enrolling by invitation</td>
<td>Migraine</td>
<td>Tehran University of Medical Sciences</td>
<td>September 2015</td>
<td>Phase 4</td>
</tr>
<tr>
<td>NCT02529992</td>
<td>Recruiting</td>
<td>Non Insulin Dependent Diabetes</td>
<td>National Nutrition and Food Technology Institute</td>
<td>July 2015</td>
<td>Phase 3</td>
</tr>
<tr>
<td>NCT02478708</td>
<td>Not yet recruiting</td>
<td>Schizophrenia/Schizoaffective Disorder</td>
<td>Yale University</td>
<td>July 2015</td>
<td>Phase 2</td>
</tr>
<tr>
<td>NCT02277223</td>
<td>Not yet recruiting</td>
<td>Ulcerative Colitis</td>
<td>Schneider Childrens Medical Center, Israel</td>
<td>July 2015</td>
<td>Phase 3</td>
</tr>
<tr>
<td>NCT02494441</td>
<td>Not yet recruiting</td>
<td>Polycystic Kidney, Autosomal Dominant</td>
<td>University of Colorado, Denver</td>
<td>July 2015</td>
<td>Phase 4</td>
</tr>
<tr>
<td>NCT02496966</td>
<td>Recruiting</td>
<td>Non Insulin Dependent Diabetes</td>
<td>National Nutrition and Food Technology Institute</td>
<td>July 2015</td>
<td>Phase 3</td>
</tr>
<tr>
<td>NCT02496882</td>
<td>Recruiting</td>
<td>Non Insulin Dependent Diabetes</td>
<td>National Nutrition and Food Technology Institute</td>
<td>July 2015</td>
<td>Phase 3</td>
</tr>
<tr>
<td>NCT02470708</td>
<td>Not yet recruiting</td>
<td>Schizophrenia/Schizoaffective Disorder</td>
<td>Yale University</td>
<td>July 2015</td>
<td>Phase 2</td>
</tr>
<tr>
<td>NCT02277223</td>
<td>Not yet recruiting</td>
<td>Ulcerative Colitis</td>
<td>Schneider Childrens Medical Center, Israel</td>
<td>July 2015</td>
<td>Phase 3</td>
</tr>
<tr>
<td>NCT02494441</td>
<td>Not yet recruiting</td>
<td>Polycystic Kidney, Autosomal Dominant</td>
<td>University of Colorado, Denver</td>
<td>July 2015</td>
<td>Phase 4</td>
</tr>
<tr>
<td>NCT02496966</td>
<td>Recruiting</td>
<td>Non Insulin Dependent Diabetes</td>
<td>National Nutrition and Food Technology Institute</td>
<td>July 2015</td>
<td>Phase 3</td>
</tr>
<tr>
<td>NCT02496882</td>
<td>Recruiting</td>
<td>Non Insulin Dependent Diabetes</td>
<td>National Nutrition and Food Technology Institute</td>
<td>July 2015</td>
<td>Phase 3</td>
</tr>
<tr>
<td>NCT02459385</td>
<td>Not yet recruiting</td>
<td>Colorectal Cancer</td>
<td>Gachon University Gil Medical Center</td>
<td>May 2015</td>
<td>Phase 2</td>
</tr>
<tr>
<td>NCT02459385</td>
<td>Not yet recruiting</td>
<td>Colorectal Cancer</td>
<td>Gachon University</td>
<td>May 2015</td>
<td>Phase 2</td>
</tr>
<tr>
<td>NCT01740323</td>
<td>Recruiting</td>
<td>Breast Cancer</td>
<td>Andrew H Miller, Emory University</td>
<td>May 2015</td>
<td>Phase 2</td>
</tr>
</tbody>
</table>
Background

Challenges

- Nomenclature:
  - Turmeric, turmeric oil
  - Curcumin, curcuminoids
  - Standardized to x% curcumin

HydroCurcumin™

**Product Description**

HydroCurcumin™ is a solubilized curcumin product generated by the HydroParticle technology. It is easily dispersible in water, so it can be conveniently used for various types of foods including beverages. In case HydroCurcumin is used for dietary supplements it will enhance the bioavailability of curcumin.

**Specification**

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Yellow powder</td>
</tr>
<tr>
<td>Curcuminoid content</td>
<td>m.t. 20%</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>N.m.t. 5%</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>N.m.t. 10 ppm</td>
</tr>
<tr>
<td>Total microbial count</td>
<td>N.m.t. 1000 cfu/g</td>
</tr>
<tr>
<td>Yeast &amp; mold</td>
<td>N.m.t. 100 cfu/g</td>
</tr>
<tr>
<td>Salmonella &amp; E.Coli</td>
<td>Negative</td>
</tr>
</tbody>
</table>
Background

Adulteration

- Indian turmeric trade types curcumin contents ranging from 2.1% to 8.6%, with an average of 4.8%.
- *Curcuma longa* L. adulterated with wild species: *Curcuma zeodaria, Curcuma malabarica* – toxicity and poor quality
- Adulterated with artificial colors – metanyl yellow
- *Saffron is adulterated with turmeric*
Background

Challenges

• Clinical Phase I studies have shown that the blood serum levels of curcumin are in the ng/mL range after oral doses of up to 8 g of curcumin, suggesting very low gastro-intestinal bioavailability

• The reasons for the low oral bioavailability of curcumin are not yet known
  – chemical instability (degradation products are vanilin, ferulic acid, feruroyl methane)
  – rapid metabolism
  – poor absorption
  – accumulation in cells of the gastro-intestinal tract
Background

Analytical Needs

• Quantitative method for curcuminoids in
  – Raw material (plant material without authentication)
  – Extracts
  – Finished products containing only turmeric and/or curcuminoids
  – Finished products containing other ingredients (vitamins, other DS, herbs)

• Quantitative method for curcuminoids in
  – Capsules
  – Tablets
  – Tinctures
  – Softgel capsules
Background

Existing Methods

• SciFinder search: “turmeric and validation” and “2014-2015” yielded 97 references

• Spectrophotometric method for the estimation of curcumin in bulk and pharmaceutical formulation

• 1H-NMR and PCR for detecting *Curcuma longa* wild species adulterants

• HPLC and LC/MS are widely used analytical techniques
Method Performance Requirements

– High and low analytical range

– Reproducibility (RSDR)
  • Original: ≤2%
  • Group discussion: ≤10%
  • After input from the industry members of the group: ≤3 and ≤6%

– Repeatability (RSDr)
  • Original: ≤1%
  • Group discussion: ≤5%
  • After input from the industry members of the group: ≤2 and ≤4%
### Dietary Supplement Label Database

(Supplement Facts Panel)

<table>
<thead>
<tr>
<th>must include</th>
<th>must include</th>
<th>products</th>
<th>&quot;Rank&quot;</th>
<th>must include</th>
<th>must include</th>
<th>products</th>
<th>&quot;Rank&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turmeric</td>
<td></td>
<td>809</td>
<td></td>
<td>Curcumin</td>
<td></td>
<td></td>
<td>300</td>
</tr>
<tr>
<td>ginger</td>
<td></td>
<td>296</td>
<td>1</td>
<td>ginger</td>
<td></td>
<td>70</td>
<td>1, 2</td>
</tr>
<tr>
<td>boswellia</td>
<td></td>
<td>88</td>
<td></td>
<td>boswellia</td>
<td></td>
<td>49</td>
<td>4</td>
</tr>
<tr>
<td>MSM</td>
<td></td>
<td>82</td>
<td></td>
<td>MSM</td>
<td></td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>glucosamine</td>
<td></td>
<td>85</td>
<td></td>
<td>glucosamine</td>
<td></td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>chondroitin</td>
<td></td>
<td>48</td>
<td></td>
<td>chondroitin</td>
<td></td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td></td>
<td>156</td>
<td>7</td>
<td>Vitamin A</td>
<td></td>
<td>47</td>
<td>6</td>
</tr>
<tr>
<td>Vitamin B</td>
<td></td>
<td>194</td>
<td>3</td>
<td>Vitamin B</td>
<td></td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>Vitamin C</td>
<td></td>
<td>289</td>
<td>2</td>
<td>Vitamin C</td>
<td></td>
<td>70</td>
<td>1, 2</td>
</tr>
<tr>
<td>Vitamin D</td>
<td></td>
<td>159</td>
<td>6</td>
<td>Vitamin D</td>
<td></td>
<td>45</td>
<td>7</td>
</tr>
<tr>
<td>Vitamin E</td>
<td></td>
<td>174</td>
<td>4</td>
<td>Vitamin E</td>
<td></td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Vitamin K</td>
<td></td>
<td>96</td>
<td></td>
<td>Vitamin K</td>
<td></td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>pepper</td>
<td></td>
<td>164</td>
<td>5</td>
<td>pepper</td>
<td></td>
<td>56</td>
<td>3</td>
</tr>
</tbody>
</table>
Workgroup meetings

Other dietary ingredients

- *Piper nigrum*
- *Zingiber officinale*
- *Capsicum annuum*
Workgroup meetings

Dietary Supplement Ingredients Absorbing in the 400-450 nm Region

- \( \alpha \)-carotene
- Antheraxanthin
- \( \beta \)-carotene
- \( \beta \)-cryptoxanthin
- Lutein
- Lycopene
- Riboflavin
- Riboflavin 5’-phosphate
- Violaxanthin
- Zeaxanthin
Dietary Supplement Ingredients Absorbing in the 400-450 nm Region

- β-carotene
- Lutein
- Lycopene
- Zeaxanthin

“For methods based on UV absorbance, all compounds in Table 2 must be evaluated for interference”
SMPR Key Points

- Reference method for cGMP compliance
- Quantitation of each individual curcuminoid and calculation of the sum of curcuminoids
- Method performance requirements:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of Quantitation (LOQ) (%)</td>
<td>≤ 0.1</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>97 – 103</td>
</tr>
<tr>
<td>Analytical Range (%)</td>
<td>≤ 0.1 – 50</td>
</tr>
<tr>
<td></td>
<td>&gt; 50</td>
</tr>
<tr>
<td>% RSD_r</td>
<td>≤ 4</td>
</tr>
<tr>
<td></td>
<td>≤ 2</td>
</tr>
<tr>
<td>% RSD_R</td>
<td>≤ 6</td>
</tr>
<tr>
<td></td>
<td>≤ 3</td>
</tr>
</tbody>
</table>
SMPR Key Points

Possible Interferences

– *Piper nigrum*
– *Zingiber officinale* (ginger)
– *Capsicum annuum* (cayenne pepper)
– β-carotene
– Lutein
– Lycopene
– Zeaxanthin
SMPR Key Points

Matrices

- Dried plant material
- Extracts (purified curcuminoids)
- Tablets
- Capsules
- Softgel capsules
- Powders
- Tinctures
- Liquids
• 1 comment was submitted

8. Validation Guidance:
  • Original text: For methods based on UV, all compounds in Table 2 must be evaluated for interference
  • Modification: For methods based on UV absorbance, all compounds in Table 2 must be evaluated for interference

• Minor editorial comments
Motion

• Move to accept the Standard Method Performance Requirements for Turmeric as presented.
Discussion?
Method Name: Quantitation of Curcuminoids

Intended Use: Reference method for cGMP compliance.

1. Purpose: AOAC SMPRs describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are written and adopted by AOAC Stakeholder Panels composed of representatives from the industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC Expert Review Panels in their evaluation of validation study data for method being considered for Performance Tested Methods or AOAC Official Methods of Analysis, and can be used as acceptance criteria for verification at user laboratories.

2. Applicability:
The method will be able to separate and quantify each individual curcuminoid, (curcumin, demethoxycurcumin, and bis-demethoxycurcumin) in turmeric [Curcuma longa Linn.] dietary ingredients and dietary supplement finished products containing turmeric, alone or in combination with other dietary ingredients.

3. Analytical Technique:
Any analytical technique(s) that measures the analytes of interest and meets the following method performance requirements is/are acceptable.

4. Definitions:

Analytes
Curcumin
IUPAC name: (1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione. CAS registry number: 458-37-7. See figure 1 for molecular structure.

Demethoxycurcumin
IUPAC name: (1E,6E)-1-(4-Hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-1,6-diene-3,5-dione. CAS registry number: 24939-17-1. See figure 2 for the molecular structure of demethoxy-curcumin.

Bisdemethoxy-curcumin
IUPAC name: (1E,6E)-1,7-Bis(4-hydroxyphenyl)hepta-1,6-diene-3,5-dione. CAS registry number: 24939-16-0. See figure 3 for molecular structure.

Dietary Ingredients
A vitamin; a mineral; an herb or other botanical; an amino acid; a dietary substance for use by man to supplement the diet by increasing total dietary intake; or a concentrate, metabolite, constituent, extract, or combination of any of the above dietary ingredients.¹

¹ Federal Food Drug and Cosmetic Act §201(ff) [U.S.C. 321 (ff)]
Dietary supplements

A product intended for ingestion that contains a "dietary ingredient" intended to add further nutritional value to (supplement) the diet. Dietary supplements may be found in many forms such as tablets, capsules, softgels, gelcaps, liquids, or powders.

Limit of Quantitation (LOQ)
The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

Quantitative method
Method of analysis which response is the amount of the analyte measured either directly (enumeration in a mass or a volume), or indirectly (color, absorbance, impedance, etc.) in a certain amount of sample.

Repeatability
Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator and repeating during a short time period. Expressed as the repeatability standard deviation (SD_r); or % repeatability relative standard deviation (%RSD_r).

Reproducibility
The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD_b); or % reproducibility relative standard deviation (%RSD_b).

Recovery
The fraction or percentage of spiked analyte that is recovered when the test sample is analyzed using the entire method.

5. Method Performance Requirements:
See table 1.

6. System suitability tests and/or analytical quality control:
Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range.

7. Reference Material(s):
Curcumin USP Reference Standard (cat no.: 1151855)
Demethoxy-curcumin USP Reference Standard (cat no.: 1173100)
Bis-demethoxy-curcumin USP Reference Standard (cat no.: 1075305)
Curcuminoids USP Reference Standard (cat no.: 1151866)
NIST SRM 3299 Curcuma longa L. (Turmeric) Rhizome
NIST SRM 3300 Curcuma longa L. (Turmeric) Rhizome Extract


8. Validation Guidance:
For methods based on UV, all compounds in Table 2 must be evaluated for interference.


109

9. **Maximum Time-To-Result**: None
Table 1: Method performance requirements.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of Quantitation (LOQ) (%)</td>
<td>≤ 0.1</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>97–103 95–110</td>
</tr>
<tr>
<td>Analytical Range (%)</td>
<td>≤ 0.1 – 50</td>
</tr>
<tr>
<td></td>
<td>&gt; 50</td>
</tr>
<tr>
<td>% RSD_r</td>
<td>≤ 4.5</td>
</tr>
<tr>
<td></td>
<td>≤ 2.3</td>
</tr>
<tr>
<td>% RSD_R</td>
<td>≤ 6.8</td>
</tr>
<tr>
<td></td>
<td>≤ 3.5</td>
</tr>
</tbody>
</table>

Table 2: Curcuminoids in the presence of other dietary ingredients, for example:

- *Piper nigrum*
- *Zingiber officinale* (ginger)
- *Capsicum annuum* (cayenne pepper)
- B-carotene
- Lutein
- Lycopene
- Zeaxanthin
- **divalent cations (calcium and magnesium) Ca+2**

Table 3: Matrices

- dried plant material
- extracts (purified curcuminoids)
- tablets
- capsules
- softgel capsules
- powders
- tinctures
- liquids
Figure 1: Molecular structure of curcumin

Figure 2: Molecular structure of demethoxycurcumin.

Figure 3: Molecular structure of bisdemethoxycurcumin
Background on Analyte

- Polysaccharides are one of the major constituents in Aloe vera.
- The major polysaccharide in aloe is glucomannan which is consisted of mannose (major) and glucose (minor) with 1,4-β-linked backbone. The mannose moieties are highly acetylated and are referred to Acemannan in literature.
Background

- Aloe leaf consists of an outer green rind (skin) and an inner clear pulp;

![Rind](Rind.png)

- Both rind and inner pulp contain polysaccharides;
- Other major components in aloe leaf are organic acids, minerals, and monosaccharides;
- Rind contains four major organic acids, malate, isocitrate, isocitrate lactone, and citrate, while pulp contains major malate and some citrate, but very minimal isocitrate and isocitrate lactone;
Background

• Based on the parts to be used, aloe leaf products can be divided into:
  – Entire leaf juice;
  – Inner leaf juice;

• Based on manufacturing process, aloe products can be divided into:
  – Enzymatic treatment;
  – Non-enzymatic treatment;

• Accepted criteria by aloe industry, the average solid contents in the inner gel are about 0.5% and entire leaf 1%.

• The concentration of aloe product is expressed:
  – 5X: 5 Time concentrated comparing with fresh aloe leaf;
  – 200X: Inner leaf extract (200 parts of inner gel to make 1 part of powder);
  – 100X: entire leaf extract (100 parts of entire leaf to make 1 part of powder) or 200X is diluted with 50% excipients;
Significance

- Acemannan is reported to be response to biological activities of *Aloe vera* including:
  - Immunostimulatory;
  - Anti-inflammatory;
  - Hypoglycemic and hypolipidemic activities;
  - Antibacterial, antiviral, and antitumor effects;

General Analytical Needs

- Method should:
  - Qualitatively identify aloe polysaccharides;
  - Quantitatively determine aloe polysaccharide contents;
  - Determine molecular weight of aloe polysaccharides;
  - Differentiate aloe product type, entire leaf vs. inner leaf;
Challenges

• A single method to meet all the requirements;
• Exclusive to aloe polysaccharides;
• Accurately quantitate aloe polysaccharides;
• Discrepancies of aloe polysaccharide structures reported in literature;

Existing Methods - General

• HPTLC
• Colorimetric Assay by Red Dye
• Colorimetric Assay of Acetyl Groups of Polysaccharides
• $^1$H NMR Spectroscopic Method
• Size Exclusion Chromatography
Existing Methods

- HPTLC

  **HPTLC conditions:**
  Plate: Si-gel Si60F254
  Reagents: anisaldehyde sulphuric acid reagent; heating the plate at 105–110°C for 5 min.

- Colorimetric Assay by Red Dye – Congo Red

  - Complex of Congo red with polysaccharides and assayed at 540 nm
Existing Methods

• Colorimetric Assay by Red Dye – Alizarin Red
  – Gu et al., Binding interaction between aloe polysaccharide and alizarin red by spectrophotometry and its analytical application. *Carbohydrate Polymers.* 2010, 80, 115–122.

\[
\text{alizarin red} + \text{Aloe polysaccharides}
\]

• Complex of alizarin red with polysaccharides and assayed at 325nm and 516nm

Existing Methods

• Colorimetric Assay of Acetyl Groups of Polysaccharides
  – Aloe Products for Food Raw Material, Chinese National Standard, QB/T 2489 2007

\[
\text{O} \quad \text{R} 
\xrightarrow{\text{NH}_2\text{OH}} 
\text{O} \quad \text{N} \quad \text{OH} 
\xrightarrow{1/3\text{Fe}^{3+}} 
\text{O} \quad \text{N} \quad \text{O} \quad \text{Fe}_3
\]

Acetohydroxamic Acid      Ferric/Acetohydroxamic Acid Complex

• The acetyl groups on polysaccharides are reacted with hydroxylamine to form acetohydroxamic acid. The resulted acetohydroxamic acid is reacted with ferric trichloride to form a ferric-acetohydroxamic acid complex and measured 540nm.
Existing Methods

• $^1$H NMR Spectroscopic Method
  
  
  
  
  
Existing Methods

- Size Exclusion Chromatography

Regulatory Guidance

- No information regarding the determination of aloe acetyl polysaccharide contents in the following pharmacopoeias:
  - United States Pharmacopeia;
  - European Pharmacopoeia
  - Chinese Pharmacopoeia;
  - Japanese Pharmacopoeia;
Fitness for Purpose (proposal)

- The methods are able to qualitatively identify aloe vera;
- Are able to accurately quantitate not only the contents of aloe polysaccharides, but also the molecular weight;
- Are able to accurately quantitate the aloe polysaccharides with different molecular weight;

QUESTIONS??
STAKEHOLDER PANEL ON Dietary Supplements
Background & Fitness for Purpose

Protein

Spencer Carter
AOAC 2016 Mid-Year Meeting
Gaithersburg, MD
17 March 2016

Outline

• Background
• Significance
• Adulteration
• Existing Methods
  • Qualitative
  • Quantitative
• Challenges with Existing Methods
• Things to Consider
• Fitness for Purpose (proposal)
• Questions
Background

• Proteins are polypeptides made of individual amino acids in a linear chain
• Form the basis of life and perform functions in every system of the human body
  • Enzymes catalyze biochemical reactions
  • Hormones are used for cell signaling and communication
  • Synthesize and repair DNA
  • Transport materials across the cell
  • Respond to stimuli
  • Provide structural support

Significance

• Estimated that 4 billion metric tons of food protein is produced globally
• Estimated that $94M was lost by changing the nitrogen-to-protein factor for dairy products from 6.38 to 6.25 in Europe in 2006
• Proteins make up $4.7B dollars in the Sports Nutrition industry, which represents 70% of the total revenue in that category
Adulteration

• Non-selective protein methods have fueled the potential to adulterate samples with non-proteins and give inaccurate results
• Melamine, urea, free amino acids cannot be differentiated using Kjeldahl, Dumas methods and have been the source of scandals
• Public health is still at risk; Economics still push adulteration

Existing Methods (Qualitative)

• Some proteins have FCC monographs. For example, Whey Protein is identified by testing for:
  – Ash
  – Fat
  – Lactose
  – Loss on drying
  – Nitrogen (and apply conversion factor)
• DNA Analysis
• LC/MS/MS
Existing Methods (Quantitative)

• Kjeldahl
  1. Wet digestion converts nitrogen to ammonium sulfate
  2. Neutralize to convert to free ammonia
  3. Distill ammonia into boric acid
  4. Back titrate with alkali
  5. Convert nitrogen concentration to protein using conversion ratio
     – “True Protein” can be determined by precipitating out protein, analyzing remaining nitrogen, subtracting from total nitrogen content

Existing Methods (con’t)

• Dumas
  1. Combust samples at high temp with oxygen to form water, carbon dioxide, nitrogen
  2. Remove water and carbon dioxide using column
  3. Nitrogen is measured using a thermal conductivity detector
  4. Convert nitrogen concentration to protein using conversion ratio
Existing Methods (con’t)

- **Amino Acids**
  1. Hydrolyze protein into amino acids
  2. Derivatize amino acids
  3. Determine protein by summing individual amino acids

- **Dye-binding**
  1. Form complex with dye and protein using ionic or electrostatic forces.
  2. Determine dye concentration using spectrophotometer

- **Copper-Binding**
  1. Copper ions react with proteins to form complex
  2. Measure absorbance at 540 nm

- **Others**
  - UV absorption
  - Infrared
Challenges with Existing Methods

- Kjeldahl, Dumas: not selective to protein;
- True Protein Kjeldahl: non-protein, nitrogen-containing compounds may precipitate or form complex with precipitated protein
- Amino Acid: Inaccurate quantitation due to variable recovery of amino acids
- Copper, Dye-Binding: other constituents besides proteins form complexes
- Lack of Standards
  - Protein biosynthesis is expensive, time-consuming, not robust
  - Proteins samples vary widely and usually include multiple proteins

Things to Consider

- Should method address qualitative or quantitative aspect? Or, both?
- Should ranges be established for quantitative methods?
- Are multiple methods required as part of orthogonal approach due to complex nature?
- How to define which proteins need to be analyzed, since samples usually contain multiple proteins, and even the same protein species can be diverse between samples?
- How to overcome the challenge of obtaining adequate reference material standards?
Fitness for Purpose (proposal)

• Qualitative method must provide positive confirmation of specific protein. Quantitative method must provide accurate and precise concentrations of specific proteins in raw materials and finished goods. Orthogonal methods may be required due to complex approach to analysis.

QUESTIONS??
STAKE HOLDER PANEL FOR DIETARY SUPPLEMENTS

Background and Fitness for Purpose

Vitamin B12

Richard B. van Breemen, Ph.D.
Gaithersburg, MD
March 17, 2016

Background on Vitamin B12

• Recognized as a fatal disease over 100 years ago, vitamin B12 deficiency causes megaloblastic anemia as well as neurological abnormalities.

• Development of effective dietary supplement therapy for “pernicious” anemia resulted in a Nobel Prize for Minot, Murphy and Whipple in 1934.

• Dorothy Hodgkin received a Nobel Prize in 1964 for her X-ray crystallographic structure determination of vitamin B12.
Background on Vitamin B12

- Vitamin B12 (cobalamin) is a group water soluble corrinoids with a cobalt-coordinated nucleotide containing the base, 5,6-dimethylbenzimidazole.
- Vitamin B12 is synthesized only in certain bacteria and becomes concentrated in higher organisms along the food chain.
- Therefore, animal-based foods are the primary sources of vitamin B12 in the human diet.
- Vegans and people with digestive insufficiencies are at greatest risk of vitamin B12 deficiency.

Vitamin B12 and Related Cobalamins

- 5'-Deoxyadenosylcobalamin and methylcobalamin are physiologically active.
- Often used in dietary supplements, other cobalamins can be converted in vivo.
- Corrinoids containing bases other than 5,6-dimethylbenzimidazole are inactive.

5'-Deoxyadenosylcobalamin
-CH₃ Methylcobalamin
-OH Hydroxocobalamin
-SO₃ Sulfocobalamin
-CN Cyanocobalamin
General Analytical Needs

- Method should
  - measure the physiologically active vitamin B12 compounds
    - 5'-deoxyadenosylcobalamin and methylcobalamin
  - measure the provitamin B12 forms
    - Including hydroxocobalamin, sulfitocobalamin and cyanocobalamin (which is the form most often used in dietary supplements)
  - distinguish between vitamin B12 active corrinoids containing the base, 5,6-dimethylbenzimidazole and inactive forms present in some dietary supplements (especially those derived from edible cyanobacteria).

Analytical Challenges

- Quantitatively extract vitamin B12 compounds from a variety of matrices including finished products such as capsules and pills and unprocessed raw materials such as cyanobacteria.
- Measurement of trace levels of vitamin B12 compounds in natural sources as well as in fortified samples.
- Measure multiple vitamin B12 compounds individually or after derivatization to a common form such as cyanocobalamin.
- Distinguish vitamin B12 cobalamins from inactive forms.
Regulations

- Intake recommendations for vitamin B12 are provided in the Dietary Reference Intakes (DRIs) developed by the Food and Nutrition Board (FNB) at the Institute of Medicine (IOM) of the National Academies (formerly National Academy of Sciences).
- For healthy adult men and women (not pregnant or lactating), the recommended daily allowance is 2.4 μg.
- RDAs for other ages: 0–6 mos 0.4 μg; 7–12 mos 0.5 μg; 1–3 yr 0.9 μg; 4–8 yr 1.2 μg; 9–13 yr 1.8 μg; 14+ yr 2.4 μg
- Prescription injectable (im), intranasal and parenteral forms of vitamin B12 are available.

Current Analytical Methods for Vitamin B12

- Bioassay using vitamin B12 dependent bacteria, such as *Lactobacillus delbrueckii* subsp. *lactis* ATCC7830
- Radioimmunoassay (RIA) and radioisotope dilution assays using radioactive $^{57}$Co & binding protein (intrinsic factor)
- Chemiluminescence using acridinium ester-labeled vitamin B12 and intrinsic factor
- Surface plasmon resonance of prepared samples
- HPLC-UV following immunoaffinity extraction
- HPLC-UV following solid phase extraction, with or without derivatization (conversion) to cyanocobalamin
- HPLC-MS and HPLC-MS/MS
Existing Methods for Vitamin B12

- USP – cyanocobalamin and hydroxocobalamin pure substances and injectable solutions, tablets and capsules by spectrophotometry and HPLC-UV
- AOAC International
  - 952.20 vitamin preparations by microbiological assay
  - 986.23 milk-based infant formula by microbiological assay
  - 2011.08 and 2011.09 infant formula and adult nutritionals by HPLC-UV with immunoaffinity extraction after conversion to cyanocobalamin (first action)
  - 2011.10 infant formula and adult nutritionals by HPLC-UV with column switching after solid phase extraction
  - 2011.16 infant formula and adult nutritionals by surface plasmon resonance

Fitness for Purpose (proposal)

The method for vitamin B12 dietary supplement analysis must quantitate multiple forms of vitamin B12 individually or after conversion to a common form (such as the more stable cyanocobalamin) in a variety of dosage forms. The method must also be able to distinguish between active vitamin B12 corrinoids and inactive forms present in products derived from some microbiological sources. As humans can only absorb 10 to 500 μg B12/day and the RDA is from 0.4 to 2.8 μg B12/day, the analytical range for supplements should extend from at least 0.1 to 1000 ppm per dosage unit.
QUESTIONS?