Attendees:

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

Darryl Sullivan, Covance (Chair)  |  Melissa Phillips, NIST
Karen Andrews, USDA               |  Tom Phillips, MD Dept. of Agriculture
Gisele Atkinson, CRN              |  Curtis Phinney, Consultant
John Austad, Covance              |  Joe Romano, Waters
Charles Barber, NIST              |  Leila Saldanha, NIH
Inger Reidun Aukrust, Kappa Biosciences |  Sushma Savarala, USDA
LaVerne Brown, NIH                |  Myron Sasser, MIDI
Anton Bzhelyansky, USP            |  Carl Schwartz, SCIEX
Jo Marie Cook, FL. Dept. of Agriculture |  Aniko Solyom, GAAS Analytical
Bob Clifford, Shimadzu             |  Douglas Stevenson, NuSkin
Stefan Gafner, American Botanical Council |  Sidney Sudberg, Alkemist
Esther Campos-Giminez, Nestlé     |  John Szpylka, Mérieux Nutrisciences
Mohamed Hamad, Micrab              |  John Travis, NSF International
Philip Haselberger, Abbott        |  Cara Welch, FDA
Kan He, Herbalife                 |  Walter Wilson, NIST
Adam Horkey, Nature’s Way         |  Laura Wood, NIST
Phil Koerner, Phenomenex          |  Jason Wubben, ADM
Greg Jaudzems, Nestlé             |  Jinchuan Yang, Waters
David Ji, Analytical Lab Anaheim / Covance |  Xionghai Yi, Shanghai CIQ
Mohamed Koroma, Pharmavite        |  Kurt Young, Nutra Manufacturing/GNC
Mary Krogull, Eurofins            |  Hong You, Eurofins
Adam Kuszak, NIH                  |  Wei Zhu, Danone
Sookwang Lee, FDA                 |  Garrett Zielinski, Covance
Vicki Manti, Danone               |  Joseph Zhou, Sunshineville Health
Katerina Mastovska, Covance       |  Jerry Zweigenbaum, Agilent
Mary McBride, Agilent             |  

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

Delia Boyd, Scott Coates, Christopher Dent, Jennifer Diatz, Arlene Fox, Dawn Frazier, Jonathan Goodwin, Nora Marshall, Deborah McKenzie, Tien Milor, Robert Rathbone, Joyce Schumacher
Meeting Minutes:

I. **Welcome and Introductions**

Sullivan opened the meeting with introductions. Sullivan announced that, effective March 10, 2017, Jim Bradford is no longer AOAC’s Executive Director. The AOAC Board of Directors has formed a search committee to find a new permanent Executive Director, however, in the interim, Jonathan Goodwin has been appointed Acting Executive Director of AOAC INTERNATIONAL. Goodwin then took the floor to explain that AOAC is moving forward faster than ever, and has eyes to continue international expansion and standards development programs. Sullivan then led introductions and the meeting commenced at approximately 8:30 a.m. ET.

II. **Sullivan Presentation**

Sullivan started with a presentation\(^1\) describing the history of the AOAC Stakeholder Panel on Dietary Supplements (SPDS), a standards development project sponsored by NIH Office of Dietary Supplements. He reviewed the status of all ingredients the panel has worked on to date, as well as the future ingredients the SPDS Advisory Panel has selected for final year of the contract. The new ingredients, selected by the SPDS Advisory Panel in December, 2016, include: Echinacea, Ginseng, SAM-e (to be launched at this meeting) and Açai, Kavalactones, and Resveratrol (to be launched in September, 2017). Scullcap was selected as an alternate, should the Advisory Panel determine that Açai is not a feasible project.

III. **Vitamin D SMPR Revision**

Sullivan invited Austad to take the floor to present the modified Vitamin D SMPR\(^2\). The only change that would be voted on was to add the words “and if possible” to the SMPR applicability statement right before “…the 25-hydroxy forms in…” This change would make separation and quantitation of the 25-hydroxy forms optional. This change was first brought to the panel’s attention at the September, 2016 SPDS Meeting and then sent out to the working group for comment. Austad said that he is present to ask for a motion to approve the revised SMPR.

McBride suggested wording that may be included in in the call for methods to make it clear that it is not expected that a single method does everything in an SMPR.

**MOTION to approve the revised AOAC SMPR.2016.016, Standard Method Performance Requirements for Determination of Vitamin D in Dietary Supplement Finished Products and Ingredients\(^1\) as presented (Zielinski / McBride).**

20 in favor, 0 opposed, 0 abstentions. The motion carried.

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\(^1\) Attachment 1 – Sullivan Background Presentation
\(^2\) Attachment 2 - Vitamin D SMPR Revision
IV. Aloe Vera SMPR Presentation

Kan He, Chair of the SPDS Working Group on Aloe Vera, took the floor to give a presentation on the work done by this group. He stated that the Stakeholder Panel reviewed the group’s first SMPR in September, 2016 and requested that the working group reconvene and develop an SMPR to include identification methods prior to putting the work up for a vote. He explained that the group did exactly that and decided to create an additional SMPR (rather than trying to combine it with the quantitative SMPR) to supplement the first, and that he would like to ask the group for a motion to approve both SMPRs.

The group had a discussion on the new Aloe Vera Identification SMPR. A member of the panel asked are there other plants with acetylated polysaccharides that might be used as adulterants? He responded that he has not seen any used as adulterants so far except for carrageenan. He also explained that the current HPLC method does can differentiate various Aloe species.

MOTION to approve the SMPR Identification of Aloe Vera in Dietary Supplements and Dietary Ingredients as presented (Travis / Sudberg).

19 in favor, 0 opposed, 0 abstentions. The motion carried.

The group then reviewed the quantitative SMPR for Aloe Vera. A member asked if 50% recovery will be a problem? He advised that this figure is only for testing the lower range. Coates then proposed a modification to the validation guidance section of the document. This section was reworded to read: Data demonstrating that the candidate method meets the performance criteria for quantitation of Aloe Vera polysaccharides in the presence adulterants listed in Table 3 and the matrices listed in Table 4 should be submitted. McBride asked for clarity regarding the term “freeze dried.” Is freeze drying the samples recommended or required? He responded that it is recommended, and that wording was also added to the SMPR.

MOTION to approve the SMPR Quantitation of Aloe Vera Characteristic Water Soluble Main Constituents in Dietary Supplements as amended at this meeting (Anderson / Bzhelyansky).

20 in favor, 0 opposed, 0 abstentions. The motion carried.

V. Ginger SMPR Presentation

Bzhelyansky, Chair of the SPDS Working Group on Ginger, then took the floor with a presentation on the work done by this group. After the presentation, he asked for a motion to approve the ginger SMPR. Prior to making a motion a minor change was made to the SMPR – Catherine Rimmer, NIST, agreed to allow the SMPR advise developers to contact her directly for reference material.

MOTION to approve the SMPR Quantitation of Select Nonvolatile Ginger Constituents as amended at this meeting (Sasser / Solyom).

3 Attachment 3 – Aloe Vera SMPR Presentation
4 Attachment 4 – Ginger SMPR Presentation
19 in favor, 0 opposed, 1 abstention (Bzhelyansky). The motion carried.

VI. **Free Amino Acids SMPR Presentation**

Zielinski, Chair of the SPDS Working Group on Free Amino Acids, took the floor to provide a presentation\(^5\) where he reviewed the work of the Free Amino Acids Working Group. He said that a few comments were submitted on this SMPR, which were mostly editorial and have been incorporated into the SMPR. Coates advised that the definition for limit of detection (LOD) should be updated and made consistent with the language in the AOAC SPIFAN Folate SMPR – ACTION for Coates to make that change prior to publication.

**MOTION to approve the SMPR for Identification and Quantitation of Free Alpha Amino Acids in Dietary Ingredients and Supplements\(^v\)** as amended at this meeting (Zielinski / Romano).

20 in favor, 0 opposed, 0 abstentions. The motion carried.

VII. **Vitamins K1 and K2 SMPR Presentation**

Reidun Aukrust, Chair of the SPDS Working Group on Vitamins K, took the floor to provide a presentation\(^6\) on the work of the group. She discussed the background, the group’s activities, and the draft standard method performance requirements developed. A member of the panel asked if 1 ppm was an appropriate low level. Reidun Aukrust replied that it is the lowest she has seen, but it does exist.

**MOTION to approve the SMPR for Determination of Vitamins K\(_1\) and K\(_2\) in Dietary Supplements and Dietary Ingredients\(^vi\)** as presented (Reidun Aukrust / Young).

20 in favor, 0 opposed, 0 abstentions. The motion carried.

VIII. **Launch: Ginseng Working Group**

Sullivan then introduced Sudberg, who has volunteered to give the launch presentation\(^7\) for the SPDS Working Group on Ginseng on behalf of working group chair, Paula Brown of British Colombia Institute of Technology not in attendance. Sullivan also advised that anyone who wants to join this or any of the other new working groups may do so via the link provided in their meeting book. Sudberg reviewed the background, significance, analytical needs, existing methods and challenges the group may face with this topic. He then proposed the following fitness for purpose statement: *Identification and quantification of the ginsenosides Rb1, Rb2, Rc, Rd, Rf Re, and Rg1 in Panax ginseng and Panax quinquifolius raw materials and finished dietary supplement materials.*

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\(^v\) Attachment 5 – Free Amino Acids SMPR Presentation
\(^vi\) Attachment 6 – Vitamins K SMPR Presentation
\(^vii\) Attachment 7 – Ginseng Launch Presentation
Sullivan asked the panel for a consensus on the proposed fitness for purpose, and the group agreed, thereby formally launching the SPDS Ginseng Working Group.

IX. **Launch: Echinacea Working Group**

Sullivan introduced Gafner, Chair of the SPDS Working Group on Echinacea. Gafner provided a presentation\(^8\) to review the background, significance, analytical needs, existing methods and challenges the group may face working on SMPRs for echinacea. He then proposed the following fitness for purpose statement: *Quantitation of phenolic compounds (i.e., caftaric acid, chlorogenic acid, cihoric acid, cynarine, and echinacoside) in Echinacea angustifolia, Echinacea pallida, and Echinacea purpurea raw materials and finished dietary supplement products.*

Kuszak then stated that this fitness for purpose statement omits identification methods. Is that the intent? The group then discussed the merits of including an SMPR for identification. Frazier highlighted that the Advisory Panel had specifically asked for quantitation methods. After further discussion, the group agreed to make an SMPR for identification methods as a secondary, optional objective. The final fitness for purpose read: **Primary objective:** *Quantitation of phenolic compounds (to include caftaric acid, chlorogenic acid, cihoric acid, cynarine, and echinacoside) in Echinacea angustifolia, Echinacea pallida, and Echinacea purpurea raw materials and finished dietary supplement products.* **Secondary Objective:** *Identification of Echinacea angustifolia, Echinacea pallida, and Echinacea purpurea raw materials and finished dietary supplement products by phenolic compound profile.*

Sullivan asked the panel for a consensus on the proposed fitness for purpose, and the group agreed, thereby formally launching the SPDS Echinacea Working Group.

X. **Launch: SAMe Working Group**

Sullivan then invited Zhou to the floor for the final launch presentation of the day to launch of the SPDS Working Group on SAMe. Zhou opened with a presentation\(^9\) describing the background of the analyte, explaining that SAMe is used as a supplement to treat depression and osteoarthritis. He then reviewed the challenges (instability), the current analytical needs, and existing methods. Finally, Zhou proposed the following fitness for purpose statement: **Methods for quantitative determination of SAMe in dietary ingredients and finished products. Method should have capability to separate SAMe from decomposition products and synthetic precursors, as well as other joint support materials.** Discussion followed the presentation. A member asked if the methods would be asked to separate the two mixtures of SAMe? Zhou said no, that is not in the current fitness for purpose. The group discussed this issue and in the end, decided to include separation as a secondary objective. The final fitness for purpose read: **Methods for the determination of SAMe in dietary ingredients and finished products. Primary objective: Method should have capability to separate SAMe from decomposition products and synthetic precursors, as well as other joint support materials. Secondary objective: Consider separation of racemic isomers.**

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\(^8\) Attachment 8 – Echinacea Launch Presentation  
\(^9\) Attachment 9 – SAMe Launch Presentation
Sullivan asked the panel for a consensus on the proposed fitness for purpose, and the group agreed, launching the SPDS SAMe Working Group.

XI. Adjourn

Sullivan advised that the group had completed their agenda for today. Sullivan thanked all for a very successful meeting – in which seven (7) SMPRs were approved and three (3) new working groups launched. Frazier also thanked all for attending and reminded them to review the “resources page” at the end of the meeting book to sign up for working groups, apply to become a method reviewer on an ERP, and/or review the open SPDS Calls for Methods. With that, the meeting adjourned at approximately 3:30pm ET.

Action Items:

- Coates to review definition of LOD in the Free Amino Acids SMPR
- Dent to pass final draft SMPRs to AOAC publications department
- ALL to review the resources page of the meeting book and consider sitting on a working group, applying to be on an Expert Review Panel, or answering an open call for methods.

List of Attachments:

1. Sullivan Background Presentation
2. Aloe Vera SMPR Presentation
3. Ginger SMPR Presentation
4. Free Amino Acids SMPR Presentation
5. Vitamins K SMPR Presentation
6. Ginseng Launch Presentation
7. Echinacea Launch Presentation
8. SAMe Launch Presentation
9. All SPDS SMPRs approved on March 17, 2017; as presented or as amended the day of

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i AOAC SMPR.2016.016, Standard Method Performance Requirements for Determination of Vitamin D in Dietary Supplement Finished Products and Ingredients as approved
ii SMPR for Identification of Aloe Vera in Dietary Supplements and Dietary Ingredients as approved
iii SMPR for Quantitation of Aloe Vera Characteristic Water Soluble Main Constituents in Dietary Supplements as approved
iv SMPR for Quantitation of Select Nonvolatile Ginger Constituents as approved
v SMPR for Identification and Quantitation of Free Alpha Amino Acids in Dietary Ingredients and Supplements as approved
vi SMPR for Determination of Vitamins K₁ and K₂ in Dietary Supplements and Dietary Ingredients as approved
Update on the Stakeholder Panel on Dietary Supplements (SPDS)

Darryl Sullivan, Chair
Stakeholder Panel on Dietary Supplements
Covance Laboratories

March 2017

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.
### Stakeholder Panel on Dietary Supplements (SPDS)

<table>
<thead>
<tr>
<th>Set 1 Ingredients: Anthocyanins, Chondroitin, and PDE5 Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Launched March, 2014</td>
</tr>
<tr>
<td>• SMPRs Approved in September, 2014:</td>
</tr>
<tr>
<td>• Authentication of Selected Vaccinium species in Dietary Ingredients and Dietary Supplements (2014.007)</td>
</tr>
<tr>
<td>• Screening Method for Selected Adulterants in Dietary Ingredients and Supplements Containing Chondroitin Sulfate (2014.008)</td>
</tr>
<tr>
<td>• Determination of Total Chondroitin Sulfate in Dietary Ingredients and Supplements (2014.009)</td>
</tr>
<tr>
<td>• Determination of Total Chondroitin Sulfate in Dietary Ingredients and Supplements (2014.009)</td>
</tr>
<tr>
<td>• Identification of Phosphodiesterase Type 5 (PDE5) Inhibitors in Dietary Ingredients and Supplements (2014.010)</td>
</tr>
<tr>
<td>• Determination of Phosphodiesterase Type 5 (PDE5) Inhibitors in Dietary Ingredients and Supplements (2014.011)</td>
</tr>
<tr>
<td>• First Action OMAs for one (1) Chondroitin and one (1) PDE5 Inhibitor method</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Set 2 Ingredients: Ashwagandha, Cinnamon, Folin C and Kratom</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Launched September, 2014</td>
</tr>
<tr>
<td>• SMPRs Approved in March, 2015:</td>
</tr>
<tr>
<td>• Withanolide Glycosides and Aglycones of Ashwagandha (2015.007)</td>
</tr>
<tr>
<td>• Alkaloids of Mitragyna speciosa (Kratom) (2015.008)</td>
</tr>
<tr>
<td>• Estimation of Total Phenolic Content Using the Folin-C Assay (2015.009)</td>
</tr>
<tr>
<td>• Identification of Selected Cinnamomum spp. Bark in Dietary Supplement Raw Materials and/or Finished Products (2015.010)</td>
</tr>
<tr>
<td>• First Action OMA for One (1) Ashwagandha Method</td>
</tr>
</tbody>
</table>

• Call for Methods and Experts currently posted for Kratom and Folin-C. Deadline is March 31, 2017. [www.aoac.org](http://www.aoac.org)
Stakeholder Panel on Dietary Supplements (SPDS)

- **Set 3 Ingredients: Aloin, Tea, and Vitamin D**
  - Launched in March, 2015
  - SMPRs Approved in September, 2015:
    - Determination of Catechins, Methyl Xanthines, Theaflavins, and Theanine in Tea Dietary Ingredients and Supplements (2015.014)
    - Determination of Aloin A and Aloin B in Dietary Supplement Products and Ingredients (2015.015)
    - Determination of Vitamin D in Dietary Supplement Finished Products and Ingredients (2015.016)
  - First Action OMAs for one (1) Aloin and one (1) Tea method
  - Determination of Vitamin D in Dietary Supplement Finished Product and Ingredients (2015.016) edits to SMPR to be recommended March 2017

- **Set 4 Ingredients: Collagen, Lutein, Turmeric**
  - Launched in September, 2015
  - SMPRs Approved in March, 2016:
    - Quantitation of Curcuminoids (2016.003)
    - Quantitative Measurement of ß-Cryptoxanthin, Lutein, and Zeaxanthin in Ingredients and Dietary Supplements (2016.004)
      Quantitation of Collagen (2016.005)
  - First Action OMAs for one (1) Curcuminoids in Turmeric Method
### Stakeholder Panel on Dietary Supplements (SPDS)

**Set 5 Ingredients: Aloe Vera, Protein, Vitamin B₁₂**
- Launched in March, 2016
- SMPRs Approved in September, 2016:
  - Identification of Proteins in Dietary Supplements
  - Identification and Quantitation of Proteins in Dietary Supplements
    - Animal Derived [2016.013](#) and Non-Animal Derived [2016.014](#)
  - Quantitative Measurement of Vitamin B₁₂ in Dietary Supplements and Ingredients [2016.017](#)
- **Call for Methods** and **Experts** will follow approval of SMPRs
- Quantitation of Aloe Vera Polysaccharides in Dietary Supplements was presented to SPDS in September, 2016 but the stakeholder panel requested additional work. Working group reconvened and developed another SMPR, *Identification of Aloe Vera in Dietary Supplements and Dietary Ingredients*.

### Stakeholder Panel on Dietary Supplements (SPDS)

**Set 6 Ingredients: Amino Acids, Ginger, Vitamins K₁ and K₂**
- Launched in September, 2016
- SMPRs sent to SPDS for approval in March, 2017:
  - Identification and Quantitation of Free Alpha Amino Acids in Dietary Ingredients and Supplements
  - Quantitation of Select Nonvolatile Ginger Constituents
  - Determination of Vitamins K₁ and K₂ in Dietary Supplements and Dietary Ingredients
- SMPR Approval Expected March, 2017
### Stakeholder Panel on Dietary Supplements (SPDS)

#### Advisory Panel

- SPDS Advisory Panel met December 2017 and recommended the last sets of ingredients for the current contract.
  - March 2017: Echinacea, Ginsenosides in Ginseng, and SAMe
  - September 2017: Amazonian Palm Fruit (Açai), Kavalactones, and Resveratrol

- Advisory Panel includes representatives from AHPA, CRN, CHPA, NSF, NPA, NIH, USP, and Herbalife

### Method Status Chart

- AOAC has prepared a Method Status Chart to keep stakeholders updated on where ingredients and methods are in process
- Methods are needed in all ingredient areas
- View the status of all submitted methods at [http://tinyurl.com/gv4w35g](http://tinyurl.com/gv4w35g)
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

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- **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

Kan He, Herbalife
Aloe Vera Working Group
March 17, 2017

Sheraton Dallas Hotel, 400 N Olive Street, Dallas, Texas

Fitness for Purpose
As Agreed March 17, 2016

“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.”
Aloe Vera Working Group Members

• John Edwards, Process NMR
• Kan He, Herbalife
• Joseph Betz, NIH
• Jasen Lavoie, Pharmachem Labs
• Barry McCleary, Megazyme
• Charles Metcalfe, Custom Analytics
• Elizabeth Mudge, BCIT
• Maria Ofitserova, Pickering Labs
• Catherine Rimmer, ATCC
• Brian Schaneberg, Starbucks
• Aniko Solyom, GAAS Analytical
• Darryl Sullivan, Covance
• Jinchaun Yang, Waters
• Kurt Young, GNC / Nutra Manufacturing

Aloe Vera Working Group
Work to Date

• 2 In Person Meeting (middle year and annual meeting 2016)

• 3 teleconferences (aloe quantitation, March 2016 – June 2016); 4 teleconferences (aloe identification, October 2016 – December 2016)

• 2 SMPR Drafted (aloe identification & quantitation)

• Public comment period (aloe quantitation, August, 2016, aloe identification, January, 2017)

• 2 SMPRs made ready for SPDS review and approval
Background

Definition:
- 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 acetylated glucomannan polysaccharides;

![Structure of the major aloe polysaccharides]

Summary of current methods used in Aloe qualification (identification) and quantitation analysis:
- $^1$H NMR
  - Qualification of aloe raw material and product;
  - Quantitation of polysaccharides by analysis of the content of acetyl groups;
  - Quantitation of organic acids including acetic acid, lactic acid, malic acid and isocitric acid;
Summary of current methods used in Aloe qualification (identification) and quantitation analysis (cont’d):

• Example of aloe identification by $^1$H NMR

![NMR spectra of Aloe whole leaf and inner gel]

Aloe whole leaf

Aloe inner gel

Unique fingerprint only found in aloe acetylated polysaccharides

• HPLC – qualification of aloe organic acid fingerprint, including malic, lactic, citric, fumaric acid, isocitric, and isocitric acid lactone. Isocitric and its lactone are whole leaf markers;

• Example of aloe HPLC fingerprint for identification;
Background

Summary of current methods used in Aloe quantitation analysis:
• $^1$H NMR – polysaccharides, monosaccharides, organic acids;
• HPLC – organic acids;
• HPAEC-PAC – organic acids, disaccharides, monosaccharide, oligosaccharides;
• GC – organic acids, monosaccharides including existed monosaccharides or hydrolyzed from polysaccharides;
• Colorimetric – quantitation of aloe polysaccharides by photometric analysis;

Summary of current methods used in Aloe quantitation analysis (cont’d):
• GPC-RI (Reflective Index)
  – Provide fingerprint of aloe polysaccharides and their molecular weight and size;
  – Require polysaccharide standards, such as dextran, pullulan;
• GPC-RI-MALLS (Multi Angle Light Scattering)
  – Measure absolute molecular weight;
  – Don’t require polysaccharide standards for quantitation;
Summary of current methods used in Aloe quantitation analysis (cont’d):

- $^1$H NMR vs. GPC-RI-MALLS
  - NMR quantitation only works on the acetylated polysaccharides;
  - Degrees of acetylation on the aloe polysaccharides are varied depending on manufacturing process;
  - GPC-RI-MALLS quantitation covers all the polymers eluted from GPC including acetylated or non-acetylated polysaccharides or other polymers such as proteins;

SMPR of Aloe Identification Key Points

- Identification of acetylated glucomannan polysaccharides derived from Aloe Vera in dietary ingredients and dietary supplements;
- Candidate methods should be able to differentiate acetylated glucomannan polysaccharides derived from whole leaf and/or inner leaf products from gel;
- Any analytical technique that meets the method performance requirements is acceptable;
- May require developing aloe polysaccharide standards for qualification;
SMPR of Aloe Identification Key Points

Selectivity

| Selectivity Study | 100% correct identification of acetylated glucomannan polysaccharides derived from *Aloe vera* in the presence or absence of potential adulterants listed in table 3.* |

*100% correct analyses are expected. Some aberrations may be acceptable if the aberrations are investigated, and acceptable explanations can be determined and communicated to method users.

SMPR of Aloe Polysaccharide Quantitation Key Points

- Quantitation of water soluble Aloe vera polysaccharides and the following organic acids (acetic acid, lactic acid, malic acid and isocitric acid) including the matrix(es) in which the polysaccharides and the acids are found);
- Any analytical technique that meets the method performance requirements is acceptable;
- It is expected that more than one technique will be required;
- May require developing aloe polysaccharide standards for quantitation;
## SMPR of Aloe Polysaccharide Quantitation Key Points

### Analytical Range & Limit of Quantitation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ingredients (Raw Materials) (1 – 100%)</th>
<th>Finished Products - Solid (1 – 100%)</th>
<th>Finished Products – Liquid (Samples to be freeze dried before analysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOQ (%)</td>
<td>≤ 0.5</td>
<td>≤ 0.5</td>
<td>≤ 0.15</td>
</tr>
<tr>
<td>Analytical Range (%)</td>
<td>1 – 100</td>
<td>1 – 100</td>
<td>0.15 – 100</td>
</tr>
</tbody>
</table>

### Recovery, Repeatability & Reproducibility

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ingredients (Raw Materials) (1 – 100%)</th>
<th>Finished Products – Solid (1 – 100%)</th>
<th>Finished Products – Liquid (Samples to be freeze dried before analysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery (%)</td>
<td>90 – 110</td>
<td>90 – 110</td>
<td>≥ 50</td>
</tr>
<tr>
<td>% RSD_r</td>
<td>≤ 10</td>
<td>≤ 10</td>
<td>≤ 20</td>
</tr>
<tr>
<td>% RSD_r</td>
<td>≤ 15</td>
<td>≤ 15</td>
<td>≤ 30</td>
</tr>
</tbody>
</table>

### Comments Submitted

- **Comment 1:** “Table 2 Recovery % is ≤ 50% for sample 0.15% - 0.5%. This would seem to want low recoveries.”;
  - **Proposed Change:** This should be ≥ 50%;
- **Comment 2:** “Tables 1 & 2: in the far right column of each table, under "liquid samples" the text "(Freeze-dried samples)". Does this include only freeze-dried samples, or is this just an example? some clarification might be useful.”;
  - **Proposed Change:** (Sample to be freeze dried before analysis);
- Other typos are corrected accordingly;
Motion

• Move to accept the Standard Method Performance Requirements for *Quantitation of Aloe Vera Polysaccharides in Dietary Supplements* as presented.

Discussion?
AOAC INTERNATIONAL
STAKEHOLDER PANEL ON
DIETARY SUPPLEMENTS
Anton Bzhelyansky, USP
Ginger Working Group - SMPR Presentation
March 17, 2017

Marriott Washingtonian Center, Gaithersburg, Maryland, USA

SPDS Ginger Working Group Members

• Anton Bzhelyansky, USP
• Gisele Atkinson, CRN
• LaVerne Brown, NIH
• Paul Burns, Eurofins
• Adam Horkey, Nature’s Way
• Holly Johnson, Alkemist Labs
• Adam Kuszak, NIH
• Andy Lippert, Weber State University
• Klaus Reif, PhytoLab GmbH & Co. KG
• Kate Rimmer, NIST
• Aniko Solyom, GAAS Analytical
• John Szypylka, Mérieux Nutrisciences
• Hong You, Eurofins
• Kurt Young, GNC / Nutra Manufacturing
Original Fitness for Purpose Statement (Working Group Launch 09/16/2016)

The method must quantitate the pungent principles derived from the rhizome of ginger, *Zingiber officinale* Roscoe. The method must quantitate, at a minimum, 6-, 8-, and 10- gingerols and 6-shogaol. The method should preferably quantitate 8- and 10- shogaols, as well as 6- and 10-paradols, 6- and 10- gingerdiols, 6-, 8-, and 10- gingerdiones and zingerone. Individual constituents should be quantifiable within the range of 0.01% and 50% by weight in powdered ginger rhizome, ginger rhizome dry and soft extracts, and ginger-containing finished products including capsules and tablets in the presence of common excipients. The ability to address softgels and tinctures is advantageous, but optional. No limit on analysis time is imposed.

Ginger Working Group Work to Date

- **In-Person Launch Meeting** (September 16, 2016 at the AOAC Annual Meeting, Dallas, TX)

- **2 Teleconferences** (October 27 & November 10, 2016)

- **1 SMPR Drafted:** *Quantitation of Select Nonvolatile Ginger Constituents*


- **SMPR is ready for SPDS review and approval**
Background

• Ginger rhizome is a widespread medicinal herb, both in the eastern and western medical traditions
• The constituents that the medicinal properties have been historically ascribed to are gingerols and shogaols; more recently, also paradols; collectively referred to as pungent principles. Quantitation of gingerdiols and gingerdiones is also conducted.
• Ginger is most commonly employed as an anti-emetic, anti-dyspeptic, anti-inflammatory, carminative, anti-thrombotic

Background

• Ginger in pharmacopoeial monographs
  – EP, BP: content of essential oil
  – JP (17 Ed.): [6]-gingerol and [6]-shogaol only for ID (TLC)
  – KP X: [6]-gingerol for ID (TLC) and assay (LC-UV)
  – ChP 2015: [6]-gingerol for ID (TLC) and assay (LC-UV)
  – USP 39: [6]-gingerol and [6]-shogaol for ID (HPTLC)
  gingerols and gingerdiones (LC-UV)
  gingerols, shogaols and gingerdiones (LC-UV)
Ginger in Other Pharmacopeial Texts

Ginger Select Nonvolatile Constituents
Availability of Ginger Reference Materials

NIST SRM 3398: Ginger (Zingiber officinale) Rhizome
Currently not for sale
NIST SRM 3399: Ginger (Zingiber officinale) Extract
Currently not for sale
USP Item # 1291504: Powdered Ginger
$369
USP Item # 1291446: Ginger Constituent Mixture
$369

Or other RMs:
Commercial Availability of Ginger Constituents

<table>
<thead>
<tr>
<th>Gingerol</th>
<th>Shogaol</th>
<th>Paradol</th>
<th>Zingerone</th>
</tr>
</thead>
<tbody>
<tr>
<td>[6]-</td>
<td>[8]-</td>
<td>[10]-</td>
<td></td>
</tr>
<tr>
<td>[6]-</td>
<td>[8]-</td>
<td>[10]-</td>
<td></td>
</tr>
<tr>
<td>[6]-</td>
<td>[8]-</td>
<td>[10]-</td>
<td></td>
</tr>
<tr>
<td>[6]-</td>
<td>[8]-</td>
<td>[10]-</td>
<td></td>
</tr>
</tbody>
</table>

Chengdu Biopurify: X X X X x
Chromadex: X x X X x
Extrasyntese: X x X X X
Phytolab: X x X x x
Sigma-Aldrich: X x X x X
Tokiwa: X x X X X
Dalton Research: X x X X X

Ginger Analytes with Chemical Identifiers

<table>
<thead>
<tr>
<th>Constituent</th>
<th>CAS Number</th>
<th>Formula</th>
<th>UNII Code</th>
<th>InChi Key</th>
<th>PubChem Link</th>
</tr>
</thead>
<tbody>
<tr>
<td>[8]-Gingerdiol</td>
<td>863780-91-0</td>
<td>C19H32O4</td>
<td>‐</td>
<td>BUACOWOGXVQEBF-VJOGAFQXNA-N</td>
<td></td>
</tr>
<tr>
<td>[8]-Gingerdiol</td>
<td>53254-76-5</td>
<td>C19H32O4</td>
<td>‐</td>
<td>RLBBNYBPCMIQMG-DLBZAZTESA-N</td>
<td></td>
</tr>
<tr>
<td>[10]-Gingerdiol</td>
<td>53254-77-6</td>
<td>C21H36O4</td>
<td>‐</td>
<td>LGSIUDXMEDKEPY-RBUKOAKNSA-N</td>
<td></td>
</tr>
<tr>
<td>[10]-Gingerdiol</td>
<td>1339934-29-0</td>
<td>C21H36O4</td>
<td>‐</td>
<td>LGSIUDXMEDKEPY-QINVSXPYNA-N</td>
<td></td>
</tr>
<tr>
<td>[10]-Gingerdione</td>
<td>79067-90-6</td>
<td>C21H32O4</td>
<td>‐</td>
<td>QPSYZJDGMPQMSV-UHFFFAOYSA-N</td>
<td></td>
</tr>
<tr>
<td>8-Paradol</td>
<td>27113-23-1</td>
<td>C19H30O3</td>
<td>‐</td>
<td>QPSYZJDGMPQMSV-UHFFFAOYSA-N</td>
<td></td>
</tr>
<tr>
<td>10-Paradol</td>
<td>36700-48-8</td>
<td>C21H34O3</td>
<td>‐</td>
<td>XNBUKRQGYHYOOP-UHFFFAOYSA-N</td>
<td></td>
</tr>
</tbody>
</table>

Note: Stereoisomers presumed to be naturally prevalent are shown in yellow.
Amongst the analyzed components 6-shogaol was found almost exclusively in the extracts of the dietary supplement ginger sample.
Analytical Methods (LC-UV)

Characteristic fingerprint based on gingerol derivative analysis for discrimination of ginger (Zingiber officinale) according to geographical origin using HPLC-DAD combined with chemometrics

1 – [6]-Gingerol
2 – Methyl [6]-gingerol
3 – [8]-Gingerol
4 – Diacetoxy-[6]-gingerdione
5 – [10]-Gingerol
6 – Acetoxy-[8]-gingerol
7 – Diacetoxy-[8]-gingerdione
8 – 1-Dehydro-[8]-gingerdione
9 – Methyl diacetoxy-[8]-gingerdione

230 nm

Vietnam ginger

Analytical Methods (LC-UV)

TN-1139

APPLICATIONS

HPLC-UV Analysis of Gingerol in Ginger Root

282 nm

AOAC SPDS Meeting Minutes
Version 3 - April 6, 2017
Analytical Methods (HPTLC, LC-UV)

Chromatographic analysis, antioxidant, anti-inflammatory, and xanthine oxidase inhibitory activities of ginger extracts and its reference compounds

Shivraj Hariram Nile*, Se Won Park

Department of Bio-Resources and Biotechnology, College of Life and Environmental Sciences, Konkuk University, Seoul 422-701, South Korea

Fig. 1. HPTLC chromatograms of the tested ginger rhizome extracts, lane assignments, from left to right: standards 1-6: rhizome, 2: ginger rhizome extract, 3: ethyl acetate extract, 4: ethyl acetate extract, 5: ethyl acetate extract, 6: ethyl acetate extract, 7: ethyl acetate extract, 8: a-butanol extract.

Fig. 2. HPLC analysis of ginger extract showing 1: ginger phenolic, 2: (+)-catechin, and 3: (-)-catechin.

Analytical Methods (LC-MS)

Identification and Quantification of Gingerols and Related Compounds in Ginger Dietary Supplements Using High-Performance Liquid Chromatography–Tandem Mass Spectrometry

Yi Tao, Wenke Li, Weizhong Huang, and Richard B. Van Breemen*

Fig. A. Chromatogram of a ginger dietary supplement showing the presence of gingerols and related compounds.

Fig. B. Tandem mass spectrometry fragmentation patterns of gingerols and related compounds.
Analytical Methods (GC-MS)

Commercially processed dry ginger (Zingiber officinale): Composition and effects on LPS-stimulated PGE₂ production

Shivanand D. Jolad a, R. Clark Lantz a,d, Guan Jie Chen a,b, Robert B. Bates d, Barbara N. Tinnermann a,b,c

Fig. 1. GC chromatogram of original crude dry ginger rhizome extract (N) of dry commercial ginger. Numbers refer to Tables 2 and 3.

Ginger Dietary Supplements in ODS DSLD

Quick Search Results

Your search for “ginger” was found in the following Label elements:
1. Product Name: 96 products found containing “ginger” in the product name
2. Dietary Ingredient Name: 141 dietary ingredients found containing “ginger” as the dietary ingredient name
3. Brand Name: 1 brands found containing “ginger” in the product brand name
4. Contacts Name: 1 contact found containing “ginger” in the product contact name
5. Anywhere: 1983 products found containing “ginger” anywhere on the label
SMPR Applicability Statement
(WG Teleconference on 11/10/2016)

The method is required to quantitate [6]-, [8]- and [10]-gingerols and [6]-shogaol in the dietary ingredients and dietary supplements listed in Table 3. It is desirable, but optional, for the method to quantitate: [8]- and [10]-shogaols, [6]-, [8]- and [10]-paradols, [6]- and [10]-gingerdiols, [6]-, [8]- and [10]-gingerdiones, and zingerone.

SMPR Summary

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical Range (%)</td>
<td>0.05 – 50</td>
</tr>
<tr>
<td>Limit of Quantitation (LOQ) (%)</td>
<td>≤ 0.05</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>90 – 107</td>
</tr>
<tr>
<td>% RSD_r</td>
<td>≤ 5</td>
</tr>
<tr>
<td>% RSD_R</td>
<td>≤ 8</td>
</tr>
</tbody>
</table>
SMR: Matrices and MTTR

Matrices:
Rhizome powder
Rhizome dry extract
Tablets containing dry extract and rhizome powder
Capsules containing dry extract and rhizome powder

Optional
Softgel capsules
Tinctures

Maximum Time-to-Result: None

Validation Guidance

- Each required analyte and each claimed optional analyte should be evaluated in all claimed matrices. For each matrix evaluated, an explicit list of analytes to which validation is applicable should be provided.
- Appendix K: Guidelines for Dietary Supplements and Botanicals; http://www.eoma.aoac.org/app_k.pdf
Public Comments

No comments were received

Motion

• Move to accept the Standard Method Performance Requirements for *Quantitation of Select Nonvolatile Ginger Constituents* as presented.
Discussion?
Identification and Quantitation of individual free α-amino acids and taurine in finished dietary supplement products, including alanine, arginine, asparagines, aspartic acid, β-alanine, cysteine, glutamic acid, glutamine, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, prolie, serine, threonine, tryptophan, tyrosine, valine, and taurine.
### SPDS Free Amino Acid Working Group Members

- Garrett Zielinski, Covance
- Gisele Atkinson, CRN
- Paul Burns, Eurofins
- Danielle Citrolo, Kyowa Hakko USA
- Holly Johnson, Alkemist
- Adam Kuszak, NIH
- Maria Ofitserova, Pickering Laboratories
- Lars Reimann, Eurofins
- Kate Rimmer, NIST
- Aniko Solyom
- John Szpylka, Mérieux NutriSciences
- Kurt Young, GNC/Nutra Manufacturing

### Free Amino Acids Working Group

#### Work to Date

- 1 In Person Meeting (September 2016)
- 2 teleconferences (October 2016 – November 2016)
- 1 SMPR Drafted
- Public comment period (January, 2017)
- SMPRs made ready for SPDS review and approval
### Background

#### Amino Acid Products

- Anti-aging
- Arthritis & Osteoporosis
- Cholesterol
- Diabetes
- Fat loss
- Healthy Skin
- Hair loss
- Menopause
- Muscle growth
- Sports Nutrition
- Sleep & Mood
- Virility

### Background

#### Amino Acid Products

- Anti-aging
- Arthritis & Osteoporosis
- Cholesterol
- Diabetes
- Fat loss
- Healthy Skin
- Hair loss
- Menopause
- Muscle growth
- Sports Nutrition
- Sleep & Mood
- Virility

#### Products with Known Adulteration
Background

Free alpha amino acids and related compounds

<table>
<thead>
<tr>
<th>AA</th>
<th>Name</th>
<th>Name</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-alanine</td>
<td>Alanine</td>
<td>Arginine</td>
<td>Asparagine</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>Cysteine</td>
<td>Cystine</td>
<td>Glutamic Acid</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Glycine</td>
<td>Histidine</td>
<td>Hydroxyproline</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Leucine</td>
<td>Lysine</td>
<td>Methionine</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Proline</td>
<td>Serine</td>
<td>Taurine</td>
</tr>
<tr>
<td>Threonine</td>
<td>Tryptophan</td>
<td>Tyrosine</td>
<td>Valine</td>
</tr>
</tbody>
</table>

SMPR Key Points

Method Performance Requirements

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Acceptable Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical Range (%)</td>
<td>0.04 - 100</td>
</tr>
<tr>
<td>LOQ (%)</td>
<td>≤0.04</td>
</tr>
<tr>
<td>Recommended LOD (%)</td>
<td>≤0.01</td>
</tr>
</tbody>
</table>

For individual free amino acid components measured.

<table>
<thead>
<tr>
<th>Ranges (%)</th>
<th>0.04 -10</th>
<th>&gt; 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery (%)</td>
<td>90 - 107</td>
<td>98 – 102</td>
</tr>
<tr>
<td>% RSD</td>
<td>≤ 5</td>
<td>≤ 3</td>
</tr>
<tr>
<td>% RSDv</td>
<td>≤ 8</td>
<td>≤ 4</td>
</tr>
</tbody>
</table>

For individual free amino acid components measured.


**Comments Submitted (if any)**

- Minor editorial comment:
  - *Free Amino* is crossed out in the title of Table 3.
  - *Free Amino Acid* is highlighted on the bottom of both Table 3 and 4.

**Motion**

- Move to accept the Standard Method Performance Requirements for *Identification and Quantitation of Free Alpha Amino Acids in Dietary Ingredients and Supplements* as presented.
Discussion?
AOAC INTERNATIONAL
STAKEHOLDER PANEL ON
DIETARY SUPPLEMENTS

Inger Reidun Aukrust, Kappa Bioscience
Vitamins K₁ and K₂ Working Group
March 17, 2017

Marriott Washingtonian Center, Gaithersburg, Maryland, USA

Fitness for Purpose
As Agreed September 16, 2016

The analytical range of the chosen method must encompass the vitamin K content in dietary supplements and their raw materials
• Dietary supplements (5-200 µg/dose), custom premixes, and raw materials 0.1 -100%

The method should:
• Separate and accurately determine both vitamin K₁ (phyllloquinone) and K₂ (different menaquinones)
• Determination of trans-K₁ and cis-K₁ (defined as the sum of cis and trans isomer of K₁)
• Separate and accurately determine three different forms of K₂ (MK₄, MK₆ and MK₇)
• Determine all trans-MK₄, all trans MK₆, and all trans MK₇. Many cis forms may be present.
• Determination of all-trans-MK₄, all-trans MK₆ and all-trans MK₇. Many cis forms maybe present.
• Be able to analyze both coated and non-coated formulations
• Determine the above in raw materials used to produce/formulate dietary supplements
### Vitamin K Working Group Members

- Inger Reidun Aukrust, Kappa Bioscience
- Gisele Atkinson, CRN
- Sneh Bhandari, Mérieux NutriSciences
- Adam Horkey, Nature's Way
- Adam Kuszak, NIH
- Elizabeth Mudge, BCIT
- Kate Rimmer, NIST
- Aniko Solyom, GAAS Analytical
- William Sommer, NattoPharma
- John Szpylka, Mérieux NutriSciences
- Hong You, Eurofins

### Vitamin K Working Group Work to Date

- 1 In Person Meeting (September 2016)
- 2 teleconferences (October 2016 – November 2016)
- 1 SMPR Drafted
- Public comment period (January, 2017)
- SMPRs made ready for SPDS review and approval
Background on Vitamin K

“Vitamin K”, the generic name for a family of compounds with a common chemical structure of 2-methyl-1,4-naphtoquinon, is a fat-soluble vitamin.

Two Primary groups:
• **Vitamin K1** (phyloquinone, defined as the sum of cis and trans isomers)
• **Vitamin K2** (the menaquinone series, MK4 through MK14).

MK4 and MK7 are the most well-studied menaquinones.

Defined as all-trans K2-MK4 and all-trans K2-MK7.

**Vitamin K1** (phyloquinone)
- Made by plants and algae-
- Only 5-10% of ingested K1 reaches circulation

**Vitamin K2 - Menaquinone 4**
- Pharmacokinetics like K1
- Used in many studies due to commercial availability

**Vitamin K2 - Menaquinone 7**
- Found in certain fermented foods
- Readily absorbed (nearly 100%) and distributed to several tissues
Background on Vitamin K

Vitamin K is an essential vitamin in many organs. Vitamin K is a necessary co-factor for activation of the Gla-proteins. Once activated, the Gla-protein can bind calcium.

Vitamin K important for:
• Blood clotting
• Building of bone (combined with calcium and vitamin D)
• Prevention of vessel calcification

The “Tri-Essentials”
Three essentials for optimal bone health

SMPR Key Points

Applicability

• Individually separate and quantify \textit{cis} and \textit{trans} forms of vitamin K1, all-trans forms of both MK4 and MK7 (vitamin K2)
• Determine area % for total \textit{cis} forms of Vitamin K2 in dietary ingredients and dietary supplements
## Matrices for vitamin K Dietary Supplements

- Powders
- Tablets
- Gummies
- Oils
- Liquids
- Capsules
- Soft gels capsules
- Tinctures
- Gelcaps
- Chewables

## Matrices for Vitamin K Dietary Ingredients

- Powders
- Oils
- Extracts
- Encapsulated
Validation Guidance


### Analytical Range & LOQ Requirements based on Matrix

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dietary Supplements</th>
<th>Dietary Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical range</td>
<td>1–3000 ppm</td>
<td>1,000 – 1M ppm</td>
</tr>
<tr>
<td>Limit of Quantitation</td>
<td>0.5 ppm</td>
<td>200 ppm</td>
</tr>
</tbody>
</table>

*Measured as individual forms of Vitamin K1 and K2 and their isomers*
Recovery, Repeatability & Reproducibility

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 – 100 ppm</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>80 – 110</td>
</tr>
<tr>
<td>% RSD,</td>
<td>&lt; 11</td>
</tr>
<tr>
<td>% RSD,</td>
<td>&lt; 15</td>
</tr>
</tbody>
</table>

* Measured as individual forms of Vitamin K1 and K2 and their isomers

Motion

- Move to accept the Standard Method Performance Requirements for *Determination of Vitamins K<sub>1</sub> and K<sub>2</sub> in Dietary Supplements and Dietary Ingredients* as presented.
Discussion?
Stakeholder Panel on Dietary Supplements: Background and Fitness for Purpose for Quantitative Determination of Selected Ginsenosides in Plant Materials, Dietary Supplements and/or Dietary Ingredients

Paula N. Brown (Rockville, MD)  
March 17, 2017

Background on the Analyte

- Ginsenosides are a secondary metabolite of interest in *Panax* sp.
- Triterpenoid saponins with most are composed of a dammarane skeleton with sugars attached at the C-3, C-6 and/or C-20 position(s).
Background on the Analyte

- Named ‘Rx’ with ‘x’ describing the chromatographic polarity in alphabetical order
- Two major classes: protopanaxadiols and protopanaxatriols.
- Protopanaxadiol have a carboxyl group at the C-6 position
- Over 100 ginsenosides have been identified
- 6 major neutral ginsenosides of interest in *Panax quinquefolius* and *Panax ginseng* are: Rb1, Rb2, Rc, Rd, Re, Rf, Rg1

---

Background on the Analyte

- *Panax* sp. also consist of significant amounts of acidic ginsenosides (also known as malonyl ginsenosides).
- Malonyl ginsenosides are unstable and can be readily converted to their neutral counterparts under typical extraction and manufacturing conditions
- Impartial hydrolysis of these compounds can affect precision and accuracy of ginsenoside quantification
Significance (or implications)

- Ginsenosides are pharmacologically active metabolites in *Panax* sp. with reported effects on the cardiovascular system, central nervous system, and immune system.

- Used as the primary marker compound for standardization of ginseng products in the market place.

- Ratios and presence/absence of specific ginsenosides can be used to differentiate species and detect adulteration with other plant parts.

General Analytical Needs

- Method should:
  - Quantify the common ginsenosides: Rb1, Rb2, Rc, Rd, Rf Re, and Rg1
  - Account for the presence of the malonyl ginsenosides to ensure consistency in testing
  - Differentiate *Panax quinquefolius* and *Panax ginseng*
  - Detect possible adulteration with leaves: ginsenoside profile differential from root.
Challenges

- Ginsenosides possess a poor chromophore limiting sensitivity achieved with UV detection
  - Despite this limitation, UV detection is preferable given the greater accessibility
  - Although mass spectral methods in published literature, ginsenosides do not not easily ionize
- Wide variety of products in marketplace
  - Different product formats
  - Combination products
  - Economic adulteration

Existing Methods (General)

HPLC with UV Detection

- SLV and Collaborative Study published in JAOAC International:
- Hydrolysis step to convert malonyl ginsenosides to their neutral counterparts to ensure consistency in testing
- Method established as fit for the purpose of determining ginsenosides in *P. ginseng* and *P. quinquefolius* roots and powdered commercial extracts.
- Method is dated, requires modernization to reduce run time
- Matrix extension to encompass broader variety of products
Existing Methods (General)

- A variety of methods have been published employing gas chromatography or liquid chromatography equipped with mass spectrometry, evaporative light scattering detection and ultraviolet detection.

- Methods describing fingerprinting techniques coupled with chemometric analyses have been reported.

- Some method target less common, but species specific ginsenosides

Proposed Fitness for Purpose

Identification and quantification of the ginsenosides Rb1, Rb2, Rc, Rd, Rf, Re, and Rg1 in *Panax ginseng* and *Panax quinquifolius* raw materials and finished dietary supplement materials.
Stakeholder Panel on Dietary Supplements: Background and Fitness for Purpose for the Quantitative Analysis of Phenolic Compounds in *Echinacea angustifolia*, *Echinacea pallida*, and *Echinacea purpurea*

Stefan Gafner
American Botanical Council
Gaithersburg, MD
March 17, 2017

Background on the Plant Material

- The genus *Echinacea* contains nine species (*E. angustifolia*, *E. atrorubens*, *E. laevigata*, *E. pallida*, *E. paradoxa*, *E. purpurea*, *E. sanguinea*, *E. simulata*, *E. tennessensis*)

- The main *Echinacea* used in commerce are as follows:
  - *Echinacea angustifolia* rhizome and root
  - *Echinacea pallida* rhizome and root
  - *Echinacea purpurea* fresh herb,
  - *Echinacea purpurea* dried herb
  - *Echinacea purpurea* rhizome and root

- Therapeutic indications include the short-term prevention and treatment of common cold (oral intake), or topically for the treatment of small superficial wounds
Background on the Plant Material (continued)

- The phytochemicals responsible for the immunostimulant properties of *Echinacea* spp. are not known.

- The following compound classes have been linked to bioactivity:
  - Alkylamides (alkamides)
  - Phenolic compounds
  - Polysaccharides
  - LPS and lipoproteins produced by bacterial endophytes

---

**Background on the *Echinacea* phenolics**

Concentrations in % of dried plant part

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Cichoric acid</th>
<th>Caftaric acid</th>
<th>Echinacoside</th>
<th>Chlorogenic acid</th>
<th>Cynarine¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. angustifolia</em></td>
<td>&lt;LOD – 0.05</td>
<td>&lt;LOD – 0.02</td>
<td>0.13 – 1.70</td>
<td>&lt;LOD – 0.15</td>
<td>0.07 – 0.34</td>
</tr>
<tr>
<td><em>E. pallida</em></td>
<td>&lt;LOD – 0.22</td>
<td>0.01 – 0.08</td>
<td>0.13 – 1.27</td>
<td>&lt;LOD – 0.30</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td><em>E. purpurea</em></td>
<td>0.33 – 2.78</td>
<td>0.35 – 0.80</td>
<td>&lt;LOD</td>
<td>&lt;LOD – 0.19</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td><em>E. purpurea</em></td>
<td>0.52 – 2.20</td>
<td>0.18 – 0.85</td>
<td>&lt;LOD</td>
<td>&lt;LOD – 0.03</td>
<td>&lt;LOD</td>
</tr>
</tbody>
</table>

¹Syn. 1,3-dicaffeoylquinic acid 
\((1R,3R,4S,5R)-1,3-bis[[((E)-3-(3,4-dihydroxyphenyl)prop-2-enyl]oxy]-4,5-dihydroxycyclohexane-1-carboxylic acid

**Background on the *Echinacea* alkylamides (alkamides, isobutylamides)**

Total alkylamide concentrations in % of dried plant part

<table>
<thead>
<tr>
<th>Alkylamides</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. angustifolia</em></td>
<td>0.01 – 0.15</td>
</tr>
<tr>
<td><em>E. pallida</em></td>
<td>not present</td>
</tr>
<tr>
<td><em>E. purpurea root</em></td>
<td>0.01 – 2.77</td>
</tr>
<tr>
<td><em>E. purpurea tops</em></td>
<td>0.02 – 0.53</td>
</tr>
</tbody>
</table>


**Significance (or implications)**

- Echinacea dietary supplement sales ranked 3rd in the conventional (mass market) channel, and 7th in the natural channel in the US in 2015
- Recent Cochrane review suggests no treatment effect, but consistently positive trends in prophylactic trials
- Echinacea adulteration: *Parthenium integrifolium*, various *Echinacea* spp., unidentified materials

General Analytical Needs

- Method should
  - Identify and quantify relevant phenolic compounds (caftaric acid, cichoric acid, chlorogenic acid, cynarine, 1,3-dicaffeoylquinic acid, echinacoside) in *Echinacea angustifolia*, *Echinacea pallida*, and *Echinacea purpurea* raw materials and a variety of dietary supplements in which echinacea (crude powdered or extracted) materials is a dietary ingredient
  - Identify *Echinacea angustifolia*, *Echinacea pallida*, and *Echinacea purpurea* adulterants in dietary supplement raw materials and finished products

Challenges

- Variety of matrixes on the market:
  - Powdered crude raw material, hydroalcoholic extracts, glycerin-water extracts, press juices
  - Combination products of echinacea with goldenseal (*Hydrastis canadensis*) and many other botanical ingredients, vitamins and minerals
- Phenolic compound stability: susceptibility to oxidation and enzymatic degradation
- Purity of standards
- Confusion in nomenclature of cynarine, and correct configuration of cynarine and 1,5-dicaffeoylquinic acid reference materials
- Transesterification of 1,5-dicaffeoylquinic acid to cynarine has been observed in artichoke (*Cynara scolymus*) after high temperature extraction
Existing Methods (General)

- Abundance of published methods, mainly using HPLC-UV or HPLC-MS
- UV/Vis spectrophotometry (Folin-Ciocalteu) used for total phenolic compounds
- HPTLC, CE-UV infrequently used

Established methods include:
- Official methods of the United States Pharmacopeia and European Pharmacopoeia
- American Herbal Pharmacopoeia
  - HPLC-UV for phenolic compounds in *Echinacea angustifolia* root
  - HPLC-UV for phenolic compounds in *Echinacea pallida* root
  - HPLC-UV for phenolic compounds in *Echinacea purpurea* root and herb
- SLV for phenolic compounds in *Echinacea angustifolia*, *Echinacea pallida*, and *Echinacea purpurea* root and herb by Brown et al. (2011)

Official Methods

- United States Pharmacopeia
  - *Echinacea angustifolia* root, powdered root, and powdered extract: HPLC-UV for phenolic compounds
  - *Echinacea pallida* root, powdered root, and powdered extract: HPLC-UV for phenolic compounds
  - *Echinacea purpurea* root, powdered root, and powdered extract: HPLC-UV for phenolic compounds

- European Pharmacopoeia
  - *Echinacea angustifolia* root (whole or cut): HPLC-UV for phenolic compounds
  - *Echinacea pallida* root (whole or cut): HPLC-UV for phenolic compounds
  - *Echinacea purpurea* root (whole or cut): HPLC-UV for phenolic compounds
  - *Echinacea purpurea* dried herb (whole or cut): HPLC-UV for phenolic compounds
Regulatory Guidance (if any)

- For dietary supplements, the relevant regulations need to be followed, e.g.,
  - Food, Drug & Cosmetic Act (FDC Act)
  - Nutrition Labeling and Education Act (NLEA) of 1990
  - Dietary Supplement Health and Education (DSHEA) Act of 1994
  - Food and Drug Administration Modernization Act (FDAMA) of 1997
  - Food Safety Modernization Act (FSMA) of 2011
- Topical echinacea products are regulated as cosmetics (claim dependent)

Proposed Fitness for Purpose

Quantitation of phenolic compounds (i.e., caftaric acid, chlorogenic acid, cichoric acid, cynarine, and echinacoside) in *Echinacea angustifolia*, *Echinacea pallida*, and *Echinacea purpurea* raw materials and finished dietary supplement products
Stakeholder Panel on Dietary Supplements: Background and Fitness for Purpose for SAMe

Joseph Zhou, Ph.D.
Sunshineville Health Products, Inc
AOAC Meeting Gaithersburg, Maryland
March 17, 2017

Background on SAMe

- SAMe Full Name: S-Adenosyl-L-Methionine;
  Other Name: SAMe, SAM-e, or SAM;
- One of the most popular dietary supplements;
- Popular Product Format: Tablets in Blister Pack;
  Dosage: 200mg-400mg/Tablet, 2-4 Tablets daily;
- Principal Structure Function: Methyl Donor
- Medical Uses: Depression, Osteoarthritis
Background on SAMe (continued)

Chemistry

- (S, S) S - Adenosyl-L-Methionine

Challenge: SAMe’s Extreme Instability

SAMe Bio-Active, (S,S) Racemization SAMe Bio-Inactive, (R,S)

Racemization

(1)

Reversible, Insignificant

Degradation

(2)

- S-adenosyl-L-homocysteine (SAH)
- Adenosine (ADE)
- Deoxy-methylthioadenosine (DMTA)
- etc...

Irreversible, Significant
Challenge: SAMe’s Extreme Instability

(1) Racemization

Positive Points about (R, S) SAMe:

- It is still SAMe;
- It is not harmful;
- It is reversible to Bioactive (S, S) SAMe;
- It is possible that (R, S) is a Time Release form of (S, S).

(2) Degradation

Loss is –permanent, Irreversible and Significant
Challenge: SAMe’s Extreme Instability

Techniques to Reduce SAMe Product Degradation

1) Chemical Method

Binding SAMe molecule with some compounds
e.g. Trehalose, Toluenesulfonic Acid

Binding sites: -COOH, -NH2, S

2) Physical Method

- Tablets - Enteric Coating
- Temperature - Refrigeration, Freezing
- Oxygen Trap

⇒ Shelf Life - Two years for current SAMe tablets
⇒ However, does not stop Racemization
General Analytical Needs

- The industry needs an accurate quantitative and qualitative analytical method to determine the amount of SAMe in the product for quality control;
- Also use the method to do product stability studies to develop a better product.

Existing Analytical Methods (General)

- Cation Exchange HPLC
  Column expensive, not accurate, hard to do;

- NMR Method
  Not for regular QC labs to use; instrument expensive, not accurate;

- UV Method
  Simple, but not accurate;

- Regular HPLC Method
  The best approach with the current analytical techniques
Existing Methods (General)

HPLC Method for Potency and Purity Test

- HPLC: Regular System
- Column: XTerra RP₈, 5µ, 4.6x 250mm
- UV Detection: 257 nm
- Mobile Phases: A: 25 mM NaH₂PO₄ buffer, B: ACN
- Features: Easy and Simple to do; Short; Low Cost; Reliable

Existing Methods (General)

A Typical Chromatogram of SAMe Tablets
Existing Methods (General)

HPLC Method for Potency and Purity Test

Some Factors about Racemization to Consider:

- Synthetic: S/R = 50/50
- Natural: 2-4 Months to S/R = 50/50
- S and R are Convertible

A Sample Chromatogram of SAMe Degradation Products
Proposed Fitness for Purpose

Methods for quantitative determination of SAMe in dietary ingredients and finished products. Method should have capability to separate SAMe from decomposition products and synthetic precursors, as well as other joint support materials.
Standard Method Performance Requirements for Determination of Vitamin D in Dietary Supplement Finished Products and Ingredients

1 Applicability
The method will separate and accurately quantitate vitamin D\textsubscript{2} (ergocalciferol), vitamin D\textsubscript{3} (cholecalciferol), and their previtamin D forms, and if possible the 25-hydroxy forms in dietary supplement finished products and the ingredients used to formulate these products. See Figure 1.

2 Analytical Technique
Any analytical technique that meets the following method performance requirements is acceptable.

3 Definitions

Dietary ingredients.—Vitamin; mineral; herb or other botanical; amino acid; 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 {United States Federal Food and Drug and Cosmetic Act §201(ff) [U.S.C. 321 (ff)]}.

Dietary supplements.—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).—Minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result

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\textsubscript{r}); or % repeatability relative standard deviation (%RSD\textsubscript{r}).

Reproducibility.—Standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD\textsubscript{R}); or % reproducibility relative standard deviation (% RSD\textsubscript{R}).

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

4 Method Performance Requirements
See Tables 1 and 2.

5 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. A control sample must be included.

6 Reference Material(s)
NIST Standard Reference Material\textsuperscript{©} 3280; the reference value of vitamin D\textsubscript{2} in NIST 3280 is 8.6 μg/g (±2.6) μg/g vitamin D\textsubscript{2}.

NIST Standard Reference Material\textsuperscript{©} 3532 D\textsubscript{3}; the reference value of vitamin D\textsubscript{3} in NIST 3532 is 1.310 ± 0.033 μg/g cholecalciferol (vitamin D\textsubscript{3}).

Figure 1. Chemical structure of vitamin D\textsubscript{2} (ergocalciferol), vitamin D\textsubscript{3} (cholecalciferol), and their previtamin D and hydroxy forms.
7 Validation Guidance


Appendix K: Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis (current edition), AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_k.pdf). Also at: J. AOAC Int. 95, 268(2012); DOI: 10.5740/jaoacint.11-447

8 Maximum Time-to-Determination

No maximum time.


<table>
<thead>
<tr>
<th>Table 1. Analytical range and LOQ based on matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>Analytical range ppm&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Limit of quantitation ppm&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Measured as individual forms of vitamin D and pre-vitamin D.

<table>
<thead>
<tr>
<th>Table 2. Method performance requirements as a function of range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Recovery, %</td>
</tr>
<tr>
<td>Repeatability (RSD&lt;sub&gt;r&lt;/sub&gt;), %</td>
</tr>
<tr>
<td>Reproducibility (RSD&lt;sub&gt;r&lt;/sub&gt;), %</td>
</tr>
</tbody>
</table>

* Measured as individual forms of vitamin D and pre-vitamin D.
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: Quantitation of total characteristic water soluble Aloe Vera main constituents and degradation products in the matrices listed in Table 4.

3. Analytical Technique: NMR, GC, Colorimetric, GPC; or any analytical technique that meets the following method performance requirements is acceptable. It is expected that more than one technique will be required.

4. Definitions:
   Aloe Vera Main Constituents and Degradation Products
   Aloe Vera Polysaccharides (Acetylated 1, 4 beta Glucomannan) is the signature component of Aloe Vera. Acetic acid is a degradation product of Aloe Vera, quantified as a measure of the level of de-acetylation of Aloe Vera polysaccharide (degradation product). Malic acid is a necessary component of Aloe Vera. Lactic acid is a product of malolactic fermentation (degradation product). Isocitrate is a marker constituent found exclusively in the plant’s outer rind and used to identify the anatomical source of the leaf material being examined.

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

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 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 tables 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. Potential Reference Material(s):
Custom Analytics (Charles Metcalfe, (803) 499-4469, cem@calabs.us) Low Molecular Weight Pure Polysaccharides (80,000 daltons)

8. Validation Guidance:
Data demonstrating that the candidate method meets the performance criteria for quantitation of aloe vera polysaccharides in the presence should be submitted for the adulterants listed in Table 3 and the matrices listed in Table 4 should be submitted.
Pharmachem Labs may provide materials for evaluation.


Appendix K: Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis (current edition), AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_k.pdf). Also at: J. AOAC Int. 95, 268(2012); DOI: 10.5740/jaoacint.11-447

9. Maximum Time-To-Result: None
### Table 1: Method performance requirements (part 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ingredients (Raw Materials)</th>
<th>Finished Products - Solid</th>
<th>Finished Products - Liquid (Freeze dried samples)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOQ (%)</td>
<td>≤ 0.5</td>
<td>≤ 0.5</td>
<td>≤ 0.15</td>
</tr>
<tr>
<td>Analytical Range (%)</td>
<td>1 – 100</td>
<td>1 – 100</td>
<td>0.15 – 100</td>
</tr>
</tbody>
</table>

*Freeze drying is recommended

### Table 2: Method performance requirements (part 2).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ingredients (Raw Materials) (1 – 100%)</th>
<th>Finished Products - Solid (1 – 100%)</th>
<th>Finished Products - Liquid (Freeze dried samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery (%)</td>
<td>90 – 110</td>
<td>90 – 110</td>
<td>≥ 50 90 – 110</td>
</tr>
<tr>
<td>% RSD₁</td>
<td>≤ 10</td>
<td>≤ 10</td>
<td>≤ 20 ≤ 10</td>
</tr>
<tr>
<td>% RSD₂</td>
<td>≤ 15</td>
<td>≤ 15</td>
<td>≤ 30 ≤ 15</td>
</tr>
</tbody>
</table>
Table 3: Potential Adulterants

Maltodextrin
Carageenan
Gum acacia
Locust gum

Table 4: List of Matrices

Tablets
Capsules
Liquids
Powders
Extracts
Plant products

f:\spds\working groups\set 5\aloe vera\smpr\aloe smpr v4.docx
Identification of Aloe Vera in Dietary Supplements and Dietary Ingredients

**Intended Use:** Reference method for cGMP compliance.

**1. Purpose:** AOAC Standard Method Performance Requirements (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:**

Identification of acetylated glucomannan polysaccharides derived from Aloe Vera in dietary ingredients as listed in Table 1 and dietary supplements as listed in Table 2. Candidate methods should be able to differentiate acetylated glucomannan polysaccharides derived from whole leaf and/or inner leaf products from gel.

**3. Analytical Technique:**

Any analytical technique that meets the method performance requirements specified in this SMPR.

**4. Definitions:**

**Acetylated glucomannan polysaccharides.**

The signature component of Aloe Vera. A polysaccharide comprising of acetylated 1,4-β-D-Glucosyl and D-Mannosyl Residues. CAS# 85507-69-3 (Aloe Vera Extract)

**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.

**5. Method Performance Requirements:**

See table 4.

¹ Federal Food Drug and Cosmetic Act §201(ff) [U.S.C. 321 (ff)]
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. Potential Reference Material(s):
Testing materials can be obtained from Charles Metcalfe, Custom Analytics.
Contact: +1(803) 499-4469 or cem@calabs.us

8. Validation Guidance:
Information on analytical performance for all claimed matrixes must be submitted. Demonstrate ability to correctly identify acetylated glucomannan polysaccharides derived from Aloe Vera from the potential adulterants listed in table 3. Validation test samples should be blind coded, and randomly mixed with respect to presence and absence of target and potential adulterants.


Appendix K: Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis (current edition), AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_k.pdf). Also at: J. AOAC Int. 95, 268(2012); DOI: 10.5740/jaoacint.11-447

Appendix N: ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods.

9. Maximum Time-To-Result: None
**Table 1: Dietary Ingredients**

<table>
<thead>
<tr>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder</td>
</tr>
<tr>
<td>concentrates</td>
</tr>
<tr>
<td>purified polysaccharides</td>
</tr>
<tr>
<td>processed polysaccharides</td>
</tr>
</tbody>
</table>

**Table 2: Dietary Supplements**

<table>
<thead>
<tr>
<th>Tablets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsules</td>
</tr>
<tr>
<td>Liquids</td>
</tr>
<tr>
<td>Powders</td>
</tr>
<tr>
<td>Extracts</td>
</tr>
<tr>
<td>Gummies</td>
</tr>
<tr>
<td>Softgels</td>
</tr>
</tbody>
</table>

**Table 3: Potential Adulterants**

| Maltodextrin |
| Carragennan  |
| Gum acacia   |
| Locust gum   |

**Table 4: Method performance requirements.**

<table>
<thead>
<tr>
<th>Selectivity Study</th>
<th>100% correct identification of acetylated glucomannan polysaccharides derived from Aloe Vera in the presence or absence of potential adulterants listed in table 3.*</th>
</tr>
</thead>
</table>

*100% correct analyses are expected. Some aberrations may be acceptable if the aberrations are investigated, and acceptable explanations can be determined and communicated to method users.
Method Name: Quantitation of Select Nonvolatile Ginger Constituents

Intended Use: Control of incoming ingredients and finished products

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 is required to quantitate [6]-, [8]- and [10]-gingerols and [6]-shogaol in the dietary ingredients and dietary supplements listed in Table 2. It is desirable, but optional, for the method to quantitate: [8]- and [10]-shogaols, [6]-, [8]- and [10]-paradols, [6]- and [10]-gingerdiones, and zingerone.

3. Analytical Technique: Any technique that quantitates the analytes defined in the Applicability statement and satisfies the method performance requirements set forth in this SMPR.

4. Definitions:

Analytes — Refer to Table 4 for the list of analytes, their chemical attributes and identifiers. Refer to Figure 1 for the chemical structures.

Dietary Ingredient — 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. Dietary ingredients are conventionally presented as powders or liquids.

Dietary supplement — A product containing a dietary ingredient intended for ingestion to supplement the diet. Dietary supplements containing dietary ingredients are commonly marketed as tablets, capsules, softgels, tinctures, or other finished dosage forms.

Limit of Quantitation (LOQ) — The minimum content of analyte in a given matrix that can be reliably and precisely quantitated in agreement with the requirements set forth in this SMPR.

Repeatability — Statistical variation in the analytical outcome arising when the maximum control over the analytical methodology is afforded. Replicate analyses are performed by the same operator within a short time period using the same instrumentation. Expressed as the repeatability standard deviation (SDr) or % repeatability relative standard deviation (%RSDr).

---

1 Federal Food Drug and Cosmetic Act §201(ff) [U.S.C. 321 (ff)]
Reproducibility — Statistical variation in the analytical outcome influenced by typical laboratory variables. Replicate analyses are conducted on different days by different operators using different sets of equipment, occasionally in different physical locations. Expressed as the reproducibility standard deviation (SDR) or % reproducibility relative standard deviation (% RSDR).

Recovery — The relative percentage of the spiked analyte recovered from a given matrix following implementation of the complete analytical procedure.

5. Method Performance Requirements:
See Table 2.

6. System suitability tests and/or analytical quality control:
Appropriate technique-specific system suitability criteria will be specified to demonstrate adequate method performance with respect to the claimed analytes.

7. Reference Material(s):
- NIST SRM 3398: Ginger (Zingiber officinale) Rhizome [in-preparation]
- NIST SRM 3399: Ginger (Zingiber officinale) Extract [in-preparation]
- USP Item # 1291504: Powdered Ginger $369
- USP Item # 1291446: Ginger Constituent Mixture $369

Or other reference materials (Kate Rimmer, NIST, Catherine.rimmer@nist.gov)

Table 1: Commercial Sources of Ginger Constituents.

<table>
<thead>
<tr>
<th>Commercially Available Ginger Constituents</th>
<th>Gingerols</th>
<th>Shogaols</th>
<th>Paradols</th>
<th>Zingerone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[6]-</td>
<td>[8]-</td>
<td>[10]-</td>
<td>[6]-</td>
</tr>
<tr>
<td>Chengdu Biopurify</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Chromadex</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Extrasynthese</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phytolab</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Sigma-Aldrich</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Tokiwa</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Dalton Research</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>


8. Validation Guidance:
Each required analyte and each claimed optional analyte should be evaluated in all claimed matrices. For each matrix evaluated, an explicit list of analytes to which validation is applicable should be provided.


9. **Maximum Time-To-Result**: None

Table 2: Method Performance Requirements.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical Range (%)</td>
<td>0.05 – 50</td>
</tr>
<tr>
<td>Limit of Quantitation (LOQ) (%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>90 – 107</td>
</tr>
<tr>
<td>% RSD&lt;sub&gt;r&lt;/sub&gt;</td>
<td>≤ 5</td>
</tr>
<tr>
<td>% RSD&lt;sub&gt;R&lt;/sub&gt;</td>
<td>≤ 8</td>
</tr>
</tbody>
</table>

Table 3: Matrices

Rhizome powder
Rhizome dry extract
Tablets or capsules containing dry extract and rhizome powder

Optional:
Rhizome soft extract
Tincture
Softgel capsules
Figure 1: Chemical Structures of Gingerols, Shogaols, Paradols, Zingerone, Gingerdiones and Gingerdiols.
Zingerone

[6]-Paradol

[8]-Paradol

[10]-Paradol

(3R,5S)-[6]-Gingerdiol

(3S,5S)-[6]-Gingerdiol

(3R,5S)-[10]-Gingerdiol

(3S,5S)-[10]-Gingerdiol
[6]-Gingerdione

[8]-Gingerdione

[10]-Gingerdione
<table>
<thead>
<tr>
<th>Compound</th>
<th>IUPAC Name</th>
<th>Formula</th>
<th>CAS Number</th>
<th>UNII Code</th>
<th>InChi Key</th>
<th>PubChem</th>
</tr>
</thead>
<tbody>
<tr>
<td>(5S)-[4]-Gingerol</td>
<td>(S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one</td>
<td>C17H26O4</td>
<td>23513-14-6</td>
<td>925Q22900</td>
<td>NLDIIKRFXEWBK-AWEZNOQCLA-N</td>
<td><a href="https://pubchem.ncbi.nlm.nih.gov/compound/442793">https://pubchem.ncbi.nlm.nih.gov/compound/442793</a></td>
</tr>
<tr>
<td>(5R)-[4]-Gingerol</td>
<td>(R)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one</td>
<td>C17H26O4</td>
<td>72749-01-0</td>
<td>L80UB138K</td>
<td>BCIWKKMTBRYQU-INIJZCTEOSA-N</td>
<td><a href="https://pubchem.ncbi.nlm.nih.gov/compound/12310197">https://pubchem.ncbi.nlm.nih.gov/compound/12310197</a></td>
</tr>
<tr>
<td>(5S)-[8]-Gingerol</td>
<td>(S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one</td>
<td>C19H30O4</td>
<td>23513-08-8</td>
<td>LB0IJB138K</td>
<td>BCIWKKMTBRYQU-MRXNPFDASA-N</td>
<td><a href="https://pubchem.ncbi.nlm.nih.gov/compound/11023711">https://pubchem.ncbi.nlm.nih.gov/compound/11023711</a></td>
</tr>
<tr>
<td>(5R)-[8]-Gingerol</td>
<td>(R)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one</td>
<td>C19H30O4</td>
<td>135272-33-2</td>
<td>BCWIJKMTBRYQU-MRXNPFDASA-N</td>
<td><a href="https://pubchem.ncbi.nlm.nih.gov/compound/168114">https://pubchem.ncbi.nlm.nih.gov/compound/168114</a></td>
<td></td>
</tr>
<tr>
<td>(5S)-[10]-Gingerol</td>
<td>(S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)tetradecan-3-one</td>
<td>C21H34O4</td>
<td>23513-15-7</td>
<td>ND6ZLI4J0V</td>
<td>AUILWNNRTYPZYAN-SFHVURJKSA-N</td>
<td><a href="https://pubchem.ncbi.nlm.nih.gov/compound/168115">https://pubchem.ncbi.nlm.nih.gov/compound/168115</a></td>
</tr>
<tr>
<td>[6]-Paradol</td>
<td>1-(4-hydroxy-3-methoxyphenyl)decan-3-one</td>
<td>C17H26O3</td>
<td>27113-22-0</td>
<td>BO24ID7E9U</td>
<td>CZNLTCTLYMLH-L-UHFFAOYSA-N</td>
<td><a href="https://pubchem.ncbi.nlm.nih.gov/compound/74378">https://pubchem.ncbi.nlm.nih.gov/compound/74378</a></td>
</tr>
<tr>
<td>[8]-Gingerdione</td>
<td>1-(4-hydroxy-3-methoxyphenyl)dodecan-3,5-dione</td>
<td>C19H28O4</td>
<td>77334-06-6</td>
<td>70E1Y63Q2L</td>
<td>Q5SRAFJNZ6KMHZ-UHFFAOYSA-N</td>
<td><a href="https://pubchem.ncbi.nlm.nih.gov/compound/14440537">https://pubchem.ncbi.nlm.nih.gov/compound/14440537</a></td>
</tr>
<tr>
<td>(3S,5R)-[10]-Gingerdiol</td>
<td>(3S,5R)-1-(4-hydroxy-3-methoxyphenyl)tetradecan-3,5-diol</td>
<td>C21H36O4</td>
<td>1339934-29-0</td>
<td>LGSJUDXMEDEKPY-QINVXSPYNA-N</td>
<td><a href="https://pubchem.ncbi.nlm.nih.gov/compound/101572265">https://pubchem.ncbi.nlm.nih.gov/compound/101572265</a></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Naturally prevalent stereoisomers are shown in bold: (5S) configuration for gingerols, (3R,5S) configuration for gingerdiols.
Identification and Quantitation of Free Alpha Amino Acids in Dietary Ingredients and 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.

2. Applicability: Methods must identify and quantify free alpha amino acids and related compounds (see Table 1) in dietary ingredients and finished dietary supplement products as listed in Table 2. May not address purity of ingredients. One or more methods may be needed to meet the entire range.

3. Analytical Technique: Any analytical technique is acceptable.

4. Definitions:

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.

Limit of Detection (LOD) The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false-positive risk and 5% false-negative risk. Replace with definition from Folate SMPR

1Federal Food Drug and Cosmetic Act §201(ff) [U.S.C. 321 (ff)]
**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 3 and 4.

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

8. **Validation Guidance:**
Data must demonstrate ability to identify and quantitate the free amino acids in Table 1 in the presence of the non-target compounds of interest in Table 5. Interferences with the identification and quantitation of target compounds should be reported in the method.

Method developers should be able to demonstrate that candidate methods can in fact identify and quantitate minor target compounds in the presence of greater concentrations of other amino acids and their related compounds.


9. **Maximum Time-To-Result:** None
### Table 1: Free alpha amino acids and related compounds

<table>
<thead>
<tr>
<th>Common name</th>
<th>IUPAC Systematic Name</th>
<th>CAS No.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-alanine</td>
<td>3-aminopropanoic acid</td>
<td>107-95-9</td>
</tr>
<tr>
<td>Alanine</td>
<td>2-aminopropanoic acid</td>
<td>302-72-7</td>
</tr>
<tr>
<td>Arginine</td>
<td>2-amino-5-(diaminomethylideneamino)pentanoic acid</td>
<td>2500-25-7</td>
</tr>
<tr>
<td>asparagine</td>
<td>2,4-diamino-4-oxobutanoic acid</td>
<td>3130-87-8</td>
</tr>
<tr>
<td>aspartic acid</td>
<td>2-aminobutanedioic acid</td>
<td>617-45-8</td>
</tr>
<tr>
<td>Cysteine</td>
<td>2-amino-3-sulfanylpropanoic acid</td>
<td>3374-22-9</td>
</tr>
<tr>
<td>Cysteine</td>
<td>2-amino-3-[[2R]-2-amino-2-carboxyethyl]disulfanyl]propanoic acid</td>
<td>923-32-0</td>
</tr>
<tr>
<td>glutamic acid</td>
<td>2-aminopentanedioic acid</td>
<td>617-65-2</td>
</tr>
<tr>
<td>glutamine</td>
<td>2,5-diamino-5-oxopentanoic acid</td>
<td>585-21-7</td>
</tr>
<tr>
<td>Glycine</td>
<td>2-aminoethanoic acid</td>
<td>56-40-6</td>
</tr>
<tr>
<td>Histidine</td>
<td>2-amino-3-(1H-imidazol-5-yl)propanoic acid</td>
<td>4998-57-6</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>4-hydroxypyrrolidine-2-carboxylic acid</td>
<td>51-35-4</td>
</tr>
<tr>
<td>isoleucine</td>
<td>2-aminomethylpentanoic acid</td>
<td>443-79-8</td>
</tr>
<tr>
<td>Leucine</td>
<td>2-amino-4-methylpentanoic acid</td>
<td>328-39-2</td>
</tr>
<tr>
<td>Lysine</td>
<td>2,6-diaminohexanoic acid</td>
<td>70-54-2</td>
</tr>
<tr>
<td>methionine</td>
<td>2-amino-4-methylsulfanylbutanoic acid</td>
<td>59-51-8</td>
</tr>
<tr>
<td>phenylalanine</td>
<td>2-amino-3-phenylpropanoic acid</td>
<td>63-91-2</td>
</tr>
<tr>
<td>Proline</td>
<td>pyrrolidine-2-carboxylic acid</td>
<td>609-36-9</td>
</tr>
<tr>
<td>Serine</td>
<td>2-amino-3-hydroxypropanoic acid</td>
<td>302-84-1</td>
</tr>
<tr>
<td>Taurine</td>
<td>2-aminoethanesulfonic acid</td>
<td>107-35-7</td>
</tr>
<tr>
<td>threonine</td>
<td>2-amino-3-hydroxybutanoic acid</td>
<td>80-68-2</td>
</tr>
<tr>
<td>tryptophan</td>
<td>2-amino-3-(1H-indol-3-yl)propanoic acid</td>
<td>54-12-6</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2-amino-3-(4-hydroxyphenyl)propanoic acid</td>
<td>556-03-6</td>
</tr>
<tr>
<td>Valine</td>
<td>2-amino-3-methylbutanoic acid</td>
<td>516-06-3</td>
</tr>
</tbody>
</table>

*CAS numbers specify the racemic forms, except for glycine and taurine which are achiral.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Acceptable Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical Range (%)</td>
<td>0.04 - 100</td>
</tr>
<tr>
<td>LOQ (%)</td>
<td>≤0.04</td>
</tr>
<tr>
<td>Recommended LOD (%)</td>
<td>≤0.01</td>
</tr>
</tbody>
</table>

For individual free amino acid components measured.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Acceptable Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranges (%)</td>
<td>0.04 - 10</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>90 - 107</td>
</tr>
<tr>
<td>% RSD_r</td>
<td>≤ 5</td>
</tr>
<tr>
<td>% RSD_R</td>
<td>≤ 8</td>
</tr>
<tr>
<td>% RSD_s</td>
<td>≤ 4</td>
</tr>
</tbody>
</table>

For individual free amino acid components measured.

Table 5: Non-target Compounds
Norvaline
Sarcosine
Carnitine
Citrulline
Ornithine
Selenomethionine
GABA
Selenocystine
5HTP
Figure 1: Molecular structures of free amino acids and related compounds identified in table 1.
Method Name: Determination of Vitamins K₁ and K₂ in Dietary Supplements and Dietary Ingredients

 Approved by: Stakeholder Panel on Dietary Supplements (SPDS)

 Intended Use:

 1. Applicability:
   Individually separate and quantify cis and trans forms of vitamin K₁ (phyllloquinone); all -
   trans forms of both MK-4 and MK-7 (vitamin K₂); and determine area % for total cis forms of
   Vitamin K₂ in dietary ingredients and dietary supplements as listed in Table 3.

 2. Analytical Technique:
   Any analytical technique that meets the following method performance requirements is
   acceptable.

 3. Definitions:

 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. [United States 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

 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
device (%RSD_{r}).

 Reproducibility. — The standard deviation or relative standard deviation calculated from
among-laboratory data. Expressed as the reproducibility relative 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.

 Vitamin K₁. — Phylloquinone. IUPAC name: 2-methyl-3-[(2E)-3,7,11,15-tetramethyl
hexadec-2-en-1-yl]naphthoquinone. CAS number: 084-80-0. See figure 1.

 Vitamin K₂. — Menaquinone with several subtypes designated as MK-n. “MK” identifies the
basic quinone ring structure and “n” designating the number of attached isoprenoid units.
See figure 1.
MK-4. — IUPAC name: 2-methyl-3-[(2E,6E,10E)-3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraen-1-yl]-1,4-Naphthalenedione
CAS number :863-61-6

MK-7. — IUPAC name: 2-[(2E,6E,10E,14E,18E,22E)-3,7,11,15,19,23,27-heptamethyloctacosa-2,6,10,14,18,22,26-heptaenyl]-3-methylnaphthalene-1,4-dione.
CAS number :2124-57-4

4. Method Performance Requirements:

Table 1: Analytical Range & LOQ Based on Matrix

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dietary Supplements</th>
<th>Dietary Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical range</td>
<td>1–3000 ppm</td>
<td>1,000 – 1M ppm</td>
</tr>
<tr>
<td>Limit of Quantitation</td>
<td>0.5 ppm</td>
<td>200 ppm</td>
</tr>
</tbody>
</table>

* Measured as individual forms of Vitamin K1 and K2 and their isomers

Table 2: Method Performance Requirements as a Function of Range

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 – 100 ppm</td>
</tr>
<tr>
<td></td>
<td>&gt;100 – 3,000 ppm</td>
</tr>
<tr>
<td></td>
<td>&gt;3,000 ppm</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>80 – 110</td>
</tr>
<tr>
<td></td>
<td>90-107</td>
</tr>
<tr>
<td></td>
<td>97 – 103</td>
</tr>
<tr>
<td>% RSD&lt;sub&gt;r&lt;/sub&gt;</td>
<td>&lt; 11</td>
</tr>
<tr>
<td></td>
<td>&lt; 6</td>
</tr>
<tr>
<td></td>
<td>&lt; 5</td>
</tr>
<tr>
<td>% RSD&lt;sub&gt;r&lt;/sub&gt;</td>
<td>&lt; 15</td>
</tr>
<tr>
<td></td>
<td>&lt; 8</td>
</tr>
<tr>
<td></td>
<td>&lt; 6</td>
</tr>
</tbody>
</table>

* Measured as individual forms of Vitamin K1 and K2 and their isomers

5. 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. A control sample must be included.

6. Reference Material(s):

NIST SRM 3280
NIST SRM 1849a
NIST SRM 3232
MK4 from Sigma Aldrich V031 Cerilliant
MK7: USP 1381119
K1: USP 1538006
K1: NIST SRM 3280 Multivitamin Tablet
7. **Validation Guidance:**

All target analytes (vitamin K₁, MK-4, and Mk-7) and all **claimed** matrixes listed in Table 3 shall be evaluated. One analyte per **claimed** matrix is acceptable provided all three analytes are represented in the complete evaluation.


8. **Maximum Time-To-Determination:** No maximum time.

**Figure 1:** Molecular structures of vitamin K₁ and K₂

```
K1

MK-4

MK-7
```
Table 3: Matrices

Dietary Ingredients:
- powders
- oils
- extracts
- encapsulated

Dietary Supplements:
- powders
- tablets
- gummies
- oils
- liquids
- capsules
- softgel capsules
- tinctures
- gelcaps
- chewables