Stakeholder Panel on Dietary Supplements

(SPDS)

Stakeholder Panel Meeting

March 16, 2018, 8:30am – 12:00pm

SPDS Chairman: Darryl Sullivan, Covance Laboratories, Madison, Wisconsin, USA

Attendees (Present During All or Part of the Meeting)

Karen Andrews, USDA  
Lei Bao, Nestlé  
Charles Barber, US NIST  
Brad Barrett, LECO Corporation  
Sneh Bhandari, Mérieux NutriSciences  
Bob Clifford, Shimadzu  
Hans Cruijisen, FrieslandCampina  
Jean-Luc Deborde, SCL France  
Steven Dentali, Dentali Botanical Sciences  
Gabriel Giancaspro, US Pharmacopeia  
Mohamed Hamad, Microbac  
Anita Hayden, New Chapter  
Sanem Hosbas Coskun, US NIST  
Holly Johnson, American Herbal Products Association  
George Joseph, AsureQuality New Zealand  
Scott Krepich, Phenomenex  
Adam Kuszak, US NIH Office of Dietary Supplements  
Chengzhu Liang, CIQ/AOAC China Section  
Katerina Mastovska, Covance  
Joanne Mayer, ADM  
Maria Ofitserova, Pickering Laboratories  
Melissa Phillips, US NIST  
Curtis Phinney, Curtis S. Phinney, CNS  
Kate Rimmer, US NIST  
Joe Romano, Waters Corporation  
Andre Santos, Agilent Technologies  
Sushma Savarala, USD5  
Sidney Sudberg, Alkemist Labs  
Darryl Sullivan, Covance  
John Szpylka, Mérieux NutriSciences  
John Travis, NSF International  
Richard van Breemen, Oregon State University  
Lindell Ward, Elanco Animal Health  
Wayne Wargo, Abbott Nutrition  
Laura Wood, US NIST  
Sudhakar Yadlapalli, First Source Laboratory Solutions, LLP  
Jinchuan Yang, Waters  
Hong You, Eurofins Scientific  
Kurt Young, GNC/Nutra Manufacturing  
Yuting Zhou, US Pharmacopeia  
Joseph Zhou, Sunshinelive Health Products  
Joyce Zhu, Jamieson Laboratories

AOAC Staff (Present During All or Part of the Meeting)

Scott Coates, Christopher Dent, Jennifer Diatz, Dawn Frazier, Jonathan Goodwin, Nora Marshall, Deborah McKenzie, La’Kia Phillips
I. Welcome and Introductions

Darryl Sullivan thanked all for attending. He led introductions and called the meeting to order at approximately 8:30 a.m. ET. Sullivan also directed all present to review the AOAC policies and procedures, which had been provided in the meeting book.

II. Ingredient Updates

Sullivan provided an update on SPDS progress to date, including the status\(^1\) of each ingredient/SMPR and Expert Review Panel (where applicable). The group discussed the ingredients and the calls for methods. It was highlighted that methods are still required from several of the ingredients for which standards have been developed. SPDS agreed that AOAC shall re-open all calls-for-methods on their website.

III. SMPR Presentation and Consensus: Kavalactones

Sullivan introduced Steven Dentali of Dentali Botanical Sciences, Chair of the Kavalactones Working Group. Dentali took the floor with a presentation\(^2\) describing the working group’s composition and activity to date, background on kavalactones, the history of Kava, and its uses. He then reviewed the key points of the draft SMPR, including its intended use, purpose, and method performance requirements. He emphasized the fact that certain flavokavains can cause illness, which is why a quantitative method for kava is so important. A member of the panel asked, would a method for just kavalactones be acceptable? Dentali stated that it would be, and this fact will be reflected in the meeting minutes. The group had agreed that this SMPR is for kavalactones, with flavokavains optional, however, members of the panel highlighted that it could really be used for either of them. The phrase “and/or” was added to the title, allowing methods for either Kavalactones and/or flavokavains to be submitted. A final sentence was also added to make it completely clear that this can be for either/or. Finally, Dentali addressed the public comments that were submitted during the public comment period.

Dentali then made a motion to approve.

MOTION to approve the Standard Method Performance Requirements for Detection of Kavalactones and/or Flavokavains from Kava (Piper methysticum) (Dentali/Giancaspro).

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

IV. SMPR Presentation and Consensus: Resveratrol

Richard van Breemen of Oregon State University and Chair of the Resveratrol Working Group took the floor with his presentation\(^3\) on the activities of the SPDS Resveratrol Working Group. He reviewed the original fitness for purpose – that the method must separate the cis and trans forms of resveratrol and quantitate the trans isomer in dietary supplements and dietary ingredients. He reviewed the working group’s composition, it’s work to date, and farther background on the analyte. He then went into detail on the SMPR’s key points, including the range, limit of quantitation, other parameters, and reference

---

1. Attachment 1: SPDS Background and Updates Presentation
2. Attachment 2: Kavalactones Presentation
3. Attachment 3: Resveratrol Presentation
materials. No comments were submitted during the public comment period. A member of the panel noticed that the limit of quantitation (LOQ) differs from the bottom of the analytical range and all agreed that this should be changed prior to approval (the change was made in real time). A few other minor corrections were made before a motion was put forward to approve.

MOTION to approve the Standard Method Performance Requirements for Determination of trans-Resveratrol in Dietary Supplements and Dietary Ingredients as amended at this meeting (Johnson/You).

21 in favor, 0 opposed, 0 abstentions. The motion passed.

V. SMPR Presentation and Consensus: Skullcap

Holly Johnson of AHPA took the floor with a presentation on the Skullcap Working Group and SMPRs. Johnson highlighted that her group developed three separate SMPRs – one for quantitation, one for identification, and one for limit tests. While it is possible that one method could do everything, it is much more likely that methods will be submitted based on a broader range of SMPRs. Johnson reviewed the working group's work to date, its membership, background of the analyte, all known species, and adulterants. She continued by discussing the key points of each SMPR. With regards to Identification of Skullcap in Raw Materials, Skullcap-based Dietary Ingredients, and Dietary Supplements, Johnson explained that this is a binary test – yes, or no. There was more discussion on this identification SMPR. It was determined that Chinese Skullcap was not adequately represented, so the aerial parts of this plant were added to Tier 2.

MOTION to approve Identification of Skullcap in Raw Materials, Skullcap-based Dietary Ingredients, and Dietary Supplements as amended at this meeting (Rimmer/Mastovska).

17 in favor, 0 opposed, 2 abstentions. The motion passed.

Johnson continued with the Limit Test for Determination of Selected Compounds from Teucrium spp. In Skullcap Materials in Commerce. After reviewing the SMPR, there was a brief discussion about what should be included in a limit test SMPR. Rimmer inquired about what guidance can we give the Expert Review Panel (ERP). There were several questions about what had to be required – does it need to quantify? Should it use the limit of quantitation (LOQ) or the limit of determination (LOD)? Is Table 4 required? Sullivan suggested that there were too many variables and that this SMPR should be returned to the working group to complete and clarify. The stakeholder panel agreed. No motion was made, but AOAC Staff will work with Johnson to schedule another meeting of the Skullcap Working Group.

For Determination of Flavonoids from Skullcap the range and LOQ were reviewed – this is a standard quantitative method. A member of SPDS asked if there are different acceptance criteria for the different species of Skullcap? Johnson said no, it will be left open to allow for Chinese skullcap as well in case there are any methods for that. Johnson then moved to approve the document.

MOTION to approve Determination of Flavonoids from Skullcap (Johnson/Yadlapalli).

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

---

4 Attachment 4: Skullcap Presentation
VI. **Next Steps**

Sullivan explained that the SMPRs that were just approved will be posted on the AOAC website with a call-for-methods to follow shortly. As discussed at this meeting, all calls-for-methods for all ingredients will be reopened to allow developers to submit methods before the contract ends on September 30. AOAC will tentatively hold ERP sessions in mid-June and also at the AOAC Annual Meeting in Toronto. Sullivan thanked all and adjourned the meeting.

**Actions:**

- AOAC Staff to re-open calls for methods for *all* ingredients.
- AOAC Staff to re-engage the Skullcap Working Group to revisit Limit Test SMPR
AOAC Stakeholder Panel on Dietary Supplements (SPDS):

Background and Updates

Darryl Sullivan, Chair
Stakeholder Panel on Dietary Supplements
Covance Laboratories

March 16, 2018

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 standard method performance requirements (SMPRs) for 25 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.
### Status of SPDS First Action Official Methods of Analysis SM Expert Review Panels (ERPs)

<table>
<thead>
<tr>
<th>Panel</th>
<th>Description</th>
</tr>
</thead>
</table>
| Aloe Vera (AOAC SMPR 2017.009, 2017.010) | - Five methods submitted, ERP TBD  
- Call for new methods remains open. |
| Aloin in Aloe (AOAC SMPR 2015.015) | - Two methods submitted, one method approved.  
- Resubmissions from last ERP will be accepted.  
- Call for new methods is closed. |
| Anthocyanins (AOAC SMPR 2014.007) | - Three methods submitted, none approved.  
- Resubmissions from last ERP will be accepted.  
- Call for new methods remains open. |
| Ashwagandha (AOAC SMPR 007) | - One method submitted, one method approved.  
- Resubmissions from last ERP will be accepted.  
- Call for new methods is closed. |
| Cinnamon (AOAC SMPR 2015.010) | - One method submitted, no methods approved.  
- Resubmissions from last ERP will be accepted.  
- Call for new methods remains open. |
| Chondroitin ERP (AOAC SMPR 2014.003) | - Four methods submitted, one approved.  
- Resubmissions from last ERP will be accepted.  
- Call for new methods is closed. |
| Collagen (AOAC SMPR 2016.005) | - No methods submitted, no methods approved.  
- Call for new methods remains open. |
| Folin-C (AOAC SMPR 2015.009) | - Four methods submitted, one method approved.  
- Accepting resubmissions from last ERP.  
- Call for new methods is closed. |
| Free Amino Acids (AOAC SMPR 2017.011) | - One method submitted, no methods approved.  
- Call for new methods is closed. |
| Ginger (AOAC SMPR 2017.013) | - One method submitted, no methods approved.  
- Accepting resubmissions from last ERP.  
- Call for new methods remains open. |
| Keratin (AOAC SMPR 2015.008) | - Four methods submitted, one method approved.  
- Accepting resubmissions from last ERP.  
- Call for new methods is closed. |
| Lutein (AOAC SMPR 2016.004) | - Two methods submitted, no methods approved.  
- Call for new methods is closed. |
| PDE5 Inhibitors (AOAC SMPRs 2014.010, 2014.011, and 2014.012) | - Five submitted, one method approved.  
- Resubmissions from last ERP will be accepted.  
- Call for Methods closed. |
- Call for methods closed. |
| Tea (AOAC SMPR 2015.014) | - Two methods submitted, one method approved.  
- Resubmissions from last ERP will be accepted.  
- Call for new methods is closed. |
| Vitamin B12 (AOAC SMPR 2016.003) | - Two methods submitted, one method approved.  
- Resubmissions from last ERP will be accepted.  
- Call for new methods is closed. |
| Vitamin D (AOAC SMPR 2015.015) | - No methods submitted, no methods approved.  
- Call for new methods remains open. |
| Vitamins K1 and K2 (AOAC SMPR 2017.013) | - No methods submitted, no methods approved.  
- Call for new methods remains open. |
NEW AND UPCOMING CALLS FOR METHODS (and Experts!):

- Echinacea (SMPR 2017.015)
- Ginseng (SMPR 2017.014)
- Kavalactones*
- Resveratrol*
- SAMe (AOAC SMPR 2017.016)
- Skullcap*

*Pending SMPR Approval

---

Stakeholder Panel on Dietary Supplements (SPDS) Advisory Panel

- SPDS Advisory Panel met in December, 2017 and confirmed the last set of ingredients for the current contract and to suggest appropriate working group Chairs.

- The Advisory Panel includes representatives from AHPA, CRN, CHPA, NSF, NPA, NIH, USP, and Herbalife
How do you get involved?

• Submit methods on the Call for Methods tab at www.aoac.org

• Volunteer for Expert Review Panels on the Call for Experts tab at www.aoac.org

• SPDS site at www.aoac.org, click “Standards”, then Stakeholder Panel on Dietary Supplements (SPDS) for complete information about the program

Contact Information

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

Brian Schaneberg, Vice Chair, SPDS
Starbucks Corporation
Email: bschaneb@starbucks.com

Contact AOAC Staff:
Tel: 301.924.7077
Web: www.aoac.org
• 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
• Christopher Dent, Standards Development Coordinator, cdent@aoac.org ext. 119
AOAC STAKEHOLDER PANEL ON
DIETARY SUPPLEMENTS

Kavalactones Working Group – SMPR Presentation
March 16, 2018

Working Group Chair:
Steven Dentali, Dentali Botanical Sciences
Marriott Washingtonian Center, Gaithersburg, Maryland, USA

SPDS Kava Working Group Members

<table>
<thead>
<tr>
<th>Steven Dentali (Chair)</th>
<th>Salvatore Parisi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cristina Amarillas</td>
<td>Klaus Reif</td>
</tr>
<tr>
<td>Tyler Blythe</td>
<td>Catherine A. Rimmer</td>
</tr>
<tr>
<td>Paula N. Brown</td>
<td>Myron Sasser</td>
</tr>
<tr>
<td>Anton Bzhelyansky</td>
<td>Aniko M. Solyom</td>
</tr>
<tr>
<td>Christine Fields</td>
<td>Jeremy Stewart</td>
</tr>
<tr>
<td>Holly E. Johnson</td>
<td>John Szpylka</td>
</tr>
<tr>
<td>Scott Krepich</td>
<td>Michael C. Tims</td>
</tr>
<tr>
<td>Adam Kuszak</td>
<td>Richard Van Breemen</td>
</tr>
<tr>
<td>Charles Metcalfe</td>
<td>Hong You</td>
</tr>
<tr>
<td>Maria Monagas</td>
<td>Hui Zhao</td>
</tr>
<tr>
<td>Elizabeth Mudge</td>
<td>Garrett Zielinski</td>
</tr>
</tbody>
</table>
SPDS Kava Working Group
Work To Date

- One in-person meeting (September 23, 2017)
- One teleconference (October 26, 2017)
- One SMPR Draft Completed
  - Determination of Kavalactones and/or Flavokavains from Kava (*Piper methysticum*)
- Public comment period (Jan 16 – Feb 26, 2018)
- SMPR made ready for SPDS review & approval

Kavalactones Begin with Kava

Kava is... a lot of things.

1. A Polynesian name for the plant known as *Piper methysticum* G. Forst. (Piperaceae), intoxicating pepper.
2. A domesticated plant cultivated in the South Pacific Islands with scores of cultivars.
3. The beverage prepared from the plant.
4. The associated ceremony.
5. Root and rhizome raw material for ingredient manufacture.
6. An ingredient for dietary supplement finished products.
7. A variety of finished dietary supplement product forms.
Kava History

- Distribution of the plant is limited to the Pacific Islands
- Its use predates local written languages (2,000-3,000 yrs est.)
- Its ritual use, central to many local cultures, was recorded by Dutch explorers in 1616 and Cook's first voyage in 1769.
- Its cultivation, use, and traditional ceremony are interwoven.
- All cultivars are sterile and related to the wild *P. wichmannii*.
- Wide variety of cultivar phenotype (color and shape appearance).
- ~120-150 known and named cultivars that are propagated based on subjective evaluation of pharmacological effects.
Kava Uses

- A natural alternative to anti-anxiety drugs
- Does not impair mental function (unlike anti-anxiety drugs)
- Sleep aid – mild sedative effects
- Relief of muscle tension or spasm due to stress
- Production of mild euphoria
- Affords mild pain relief
- May increase sociability
Kava Chemistry

Bioactive constituents are known
- Contains six major “kavalactones” (pyrones)
  - arylethylene-α-pyrenes (also chalcones and other flavones, and conjugated diene ketones)
- At least a dozen other kavalactones identified
- Constituent composition varies among cultivars
- Relationship exists between “chemotype” and traditional status
- Kava lipid soluble resin forms an emulsion (milk) in water
Kava Rituals

Kava Pharmacology

- Pharmacology depends on kavalactone make up, not plant’s visual appearance
- Only known anxiolytic substance w/o causing impairment of mental function
- Possibly also direct muscle relaxant action
- High percentage kavain and low percentage dihydrokavain & dihydromethysticin traditionally preferred
SMPR Key Points: Kava

- **Intended Use:** For quality assurance and compliance to current good manufacturing practices.
- **Purpose:** To describe the minimum recommended performance characteristics to be used during the evaluation of a method.
  - To be used by AOAC Expert Review Panels in their evaluation of validation study data for methods being considered for *Performance Tested Methods* or *AOAC Official Methods of Analysis*, and can be used as acceptance criteria for verification at user laboratories.
**SMPR Key Points: Kava**


**SMPR Key Points: Kava**

- Need to know strength of raw materials, ingredients, and finished products
  - Quantitate individual kavalactones, list in order of predominance
- Perceived need to determine cultivar type of starting material (noble cultivar vs. two-day/tudei)
  - Quantitate amounts of individual six major kavalactones
  - Different cultivars are regulated differently in Vanuatu
- Concern for controlling/limiting amount of suspected hazard
  - Limits for flavokavain B (< 2 mg/g) have been proposed.*

The method identifies and quantifies the six primary kavalactones derived from the underground portions of kava (Piper methysticum), namely desmethoxyyangonin, dihydrokavain, yangonin, kavain, dihydromethysticin, and methysticinin in plant material, dietary ingredients and dietary supplements. The method identifies and quantifies flavokavains A, B, and C in kava plant material, dietary supplements and dietary ingredients.

Test results can be used in chemotype identification but this specific determination is outside the method scope. Individual kavalactones should be quantifiable within the range of 0.1 to 50 percent by weight in forms that include liquid, soft, and dry extracts as well as in softgels, capsules, and tablets in the presence of common excipients. The ability to address kavalactones in beverages is an advantage but not a requirement. No limit on analysis time is imposed.”
Kava Materials

- Whole or powdered rootstock (root, rhizome, stump, laterals)
  - 3%-20% dry weight kavalactones in underground plant material
- Traditional non-fermented drink (more than 1,000 yrs of use)
  - ~250-300 mg kavalactones per serving
- Liquid, soft, and dry extracts: 30%-90% kavalactones
- Capsules or tablets: 50-250 mg kavalactones per serving
- Tea bags, Instant powdered drink mix
- In combination with other ingredients
- DSILD: 42 named products with 83 w/kava somewhere on label
- 2016 ABC Market Report, Natural Channel, #29, $3.2 mil, 10.3% increase over 2015 sales
Kava Industry-Interest Group Testing

- **American Kava Association**
  [http://americankavaassociation.org/](http://americankavaassociation.org/)
  - Kava growers, manufacturers, distributors, retailers and consumers who advocate for the safe and responsible distribution of kava in the United States.
  - Sets minimum quality control standards for the distribution of Kava in North America through lab testing and responsible marketing bylaws. HPLC and HPTLC mentioned.

- **True Kava** [http://www.truekava.com/](http://www.truekava.com/)
  - A non-profit corporation dedicated to the traditional use of kava
    - HPLC: Identification, purity, strength, chemotype. Method: USP Piper methysticum Root and Rhizome Powder
    - Qualitative: Indicator of noble/two day, adulteration of noble kava with two day. Method: Colorimetric assessment of kava quality, Lebot 2017

- **Individual companies and contract labs also test!**

---

SMPR Key Points: Kava

- Need to cost effectively addressing stakeholder needs
- Focus methods solely on kavalactone quantitation or include other considerations for kava quality control?
  - Deciding to accept methods that may help differentiate cultivars (noble vs. two day/tudei) based on chemotype or presence of flavokavains A, B, and C
  - Include qualitative methods?
- Obtain sufficient stakeholder engagement to best understand needs of the whole kava industry supply chain
- Inform WHO Proposal to develop a Regional Codex Standard for Kava Products for Use as a Beverage?
- Australian government funding literature safety review to determine support for Vanuatu ban on non-noble cultivars.
  - Chemical analysis important to consider when attempting to differentiate tudei from noble chemotypes.
Method Performance Requirements: Kava

**Applicability:** Identification and quantitation of the six major kavalactones and flavokavains A, B, and C derived from the underground portions of kava (*Piper methysticum*) in plant material, dietary ingredients and dietary supplements (dried plant material, liquid extracts including tinctures, soft extracts, dry extracts, tablets, and capsules including softgels).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acceptance Criteria</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kavalactones*</td>
<td>Flavokavains*</td>
</tr>
<tr>
<td><strong>Analytical Range (mg/g)</strong></td>
<td>5 – 750</td>
<td>0.1 – 25</td>
</tr>
<tr>
<td><strong>Limit of Quantitation (mg/g)</strong></td>
<td>≤ 5</td>
<td>≤ 0.1</td>
</tr>
</tbody>
</table>

*Reported as individual constituents.

**Range may be narrower depending on the analytical matrix.**
SMPR Comments & Responses

- The purities of the standards specified should be evaluated by both HPLC and UV Absorption (related \( \varepsilon \)). The related UV \( \varepsilon \) could be confirmed or validated for all the standards and could also be used to determine the purities or concentrations. This will ensure that the standards pushed out will be equivalent and reduce variation between labs.
  - Consider adding standard purity validation step to process.

- Generally applicable to use of reference materials.
- Outside the scope of individual SMPRs.

---

SMPR Comments & Responses

- The definition of analytical range (*Includes all steps of the analytical procedure including sample preparation and further dilutions*) is a description, not a definition.
  - Issue is not limited to this SMPR. An appropriate definition is requested. Proposed: The range range of concentration of analyte that the method must adequately determine.

- Lower and upper range for flavokavins is not specified. Recommended to have defined range for components, maybe lower range of 0.1-5 and higher range of 5-25 (mg/g) to align with kavalactone column.
  - Comments on method performance req. Function-of-Range table. Flavokavain range specified as 0.1 – 25 mg/g in previous table.
  - The working group agreed on different analytical ranges for kavalactones and flavokavains.
Motion to Approve Kava SMPR

Move to approve the Standard Method Performance Requirements (SMPRs) for Determination of Kavalactones and/or Flavokavains from Kava (Piper methysticum).

More Discussion?
Thank you for your attention!
AOAC STAKEHOLDER PANEL ON
DIETARY SUPPLEMENTS

Resveratrol Working Group – SMPR Presentation
March 16, 2018

Working Group Chair:
Richard B. van Breemen, Oregon State University
Marriott Washingtonian Center, Gaithersburg, Maryland, USA

Fitness for Purpose
As Agreed September 22, 2017

The method must separate the cis and trans forms of resveratrol and quantitate the trans isomer in dietary supplements and dietary ingredients.
SPDS Resveratrol Working Group Members

Richard van Breemen, Oregon State University  
Klaus Reif, PhytoLab GmbH & Co.  
Anton Bzhelyansky, US Pharmacopeia  
Catherine A. Rimmer, NIST  
Nour Eddine Es-Safi, Mohammad V University  
Aniko M. Solyom, GAAS Analytical  
Martha Jennens, Covance  
Jeremy Stewart, Gaia Herbs  
Holly E. Johnson, AHPCA  
John Szpylka, Mérieux NutriSciences  
Scott Krepich, Phenomenex  
Hong You, Eurofins  
Adam Kuszak, NIH ODS  
Kurt Young, GNC/Nutra Manufacturing  
Maria Monagas, US Pharmacopeia  
Hui Zhao, Covance  
Garrett Zielinski, Covance

SPDS Resveratrol Working Group

Work To Date

• 1 in-person meeting (September 23, 2017)
• 1 teleconference (November 3, 2017)
• 1 SMPR Draft Completed  
  • *Determination of trans-Resveratrol in Dietary Supplements and Dietary Ingredients*
• Public comment period (January - February, 2018)
• SMPR made ready for SPDS review and approval
Background: Resveratrol

**Trans-Resveratrol (C\textsubscript{14}H\textsubscript{12}O\textsubscript{3})**

3,5,4′-trihydroxy-trans-stilbene

CAS number: 501-36-0  
IUPAC name:  
5-[(E)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol

First isolated in 1939 from *Veratrum album* by Michio Takaoka

\(J. \text{Chem. Soc. Japan} 1939; 60: 1090–1100\)

Background: Resveratrol

**Resveratrol cis/trans Chemistry**

Although trans-resveratrol is the biosynthetic product, UV exposure can isomerize it to the cis isomer.
Background: Resveratrol

Resveratrol Bioactivities

In 1997, Pezzuto (Science, 1987; 275: 218) discovered that resveratrol from grapes, berries and other sources had cancer chemoprevention activity through multiple mechanisms of action

- anti-inflammation (inhibits COX, iNOS, and NF-κB)
- anti-oxidation (upregulates quinone reductase, glutathione, superoxide dismutase, and catalase)
- induction of apoptosis

Subsequently, >20,000 papers have reported multiple other activities including

- prevention of cardiovascular disease
- anti-aging
- neuroprotection

SMPR Key Points: Resveratrol

- The method must be specific for trans-resveratrol in the presence of the cis isomer in dietary supplements and dietary ingredients
- Examples of dietary supplements and dietary ingredients containing resveratrol include
  - Powders
  - Tablets
  - Capsules
  - Liquids
  - Softgels
  - Extracts
- Reference materials are available from multiple sources for trans-resveratrol, [d₄]-trans-resveratrol, and cis-resveratrol
Method Performance Requirements: Resveratrol

Table 2: Analytical Range & LOQ Based on Matrix

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical range (% w/w)</td>
<td>0.05 - 100</td>
</tr>
<tr>
<td>Limit of Quantitation (% w/w)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Range may be narrower depending on the analytical matrix.*

Table 3: Method Performance Requirements as a Function of Range

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1%</td>
<td>1-50%</td>
</tr>
<tr>
<td>85 - 115</td>
<td>97 - 103</td>
</tr>
<tr>
<td>98 - 102</td>
<td></td>
</tr>
<tr>
<td>≤ 7.5</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>≤ 10</td>
<td>≤ 8</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

SMPR Comments & Responses

- *No comments submitted*
Motion to Approve Resveratrol SMPR

Move to approve the Standard Method Performance Requirements (SMPRs) for Determination of trans-Resveratrol in Dietary Supplements and Dietary Ingredients

Discussion?
AOAC STAKEHOLDER PANEL ON
DIETARY SUPPLEMENTS

Skullcap Working Group – SMPR Presentation
March 16, 2018

Working Group Chair:
Holly Johnson, American Herbal Products Association
Marriott Washingtonian Center, Gaithersburg, Maryland, USA

Fitness for Purpose
As Agreed September 23, 2017

Quantitative SMPR:-
Identification and quantitation of select flavonoids
(baicalin, baicalein, wogonin, wogonoside,
scutellarein) from skullcap (scullcap) in plant material,
dietary ingredients and dietary supplements.

Identification SMPR:-
Detection of constituents of *Teucrium canadense* and
*T. chamaedrys* in plant material, dietary ingredients
and dietary supplements being represented as
*Scutellaria lateriflora*. 
SPDS Skullcap Working Group Members

<table>
<thead>
<tr>
<th>Name</th>
<th>Organization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holly E. Johnson</td>
<td>American Herbal Products Association</td>
</tr>
<tr>
<td>Cristina Amarillas,</td>
<td>Traditional Medicinals</td>
</tr>
<tr>
<td>Anton Bzhelyansky</td>
<td>US Pharmacopeia (USP)</td>
</tr>
<tr>
<td>Martha Jennens</td>
<td>Covance Laboratories</td>
</tr>
<tr>
<td>Scott Krepich</td>
<td>Phenomenex</td>
</tr>
<tr>
<td>Adam Kuszak</td>
<td>NIH Office of Dietary Supplements</td>
</tr>
<tr>
<td>Maria Monagas</td>
<td>US Pharmacopeia</td>
</tr>
<tr>
<td>Salvatore Parisi</td>
<td>COIF Association, Italy</td>
</tr>
<tr>
<td>Klaus Reif</td>
<td>PhytoLab GmbH &amp; Co., KG</td>
</tr>
<tr>
<td>Catherine A. Rimmer</td>
<td>NIST</td>
</tr>
<tr>
<td>Jeremy Stewart</td>
<td>Gaia Herbs, Inc</td>
</tr>
<tr>
<td>John Szpylka</td>
<td>Mérieux NutriSciences</td>
</tr>
<tr>
<td>Michael C. Tims</td>
<td>NIST</td>
</tr>
<tr>
<td>Richard Van Breemen</td>
<td>Oregon State University</td>
</tr>
<tr>
<td>Hui Zhao</td>
<td>Covance</td>
</tr>
</tbody>
</table>

SPDS Skullcap Working Group
Work To Date

• 1 in-person meeting (September 23, 2017)

• 4 teleconferences (October 2017 – January 2018)

• 3 SMPRs Draft Completed
  • Determination of Select Flavanoids from Skullcap
  • Identification of Skullcap in Raw Materials, Skullcap-based Dietary Ingredients, and Dietary Supplements
  • Limit Test for Determination of Selected Compounds from Teucrium spp. In Skullcap Materials in Commercs

• Public comment period (January - February, 2018)

• SMPRs made ready for SPDS review and approval
Skullcap (*Scutellaria lateriflora* L.)

- Aerial parts used in modern western herbalism as a nervine, sedative, anxiolytic
- First described in Tournefort’s *The Compleat Herbal* (1719)
- Given current name by Linneaus in 1753
Skullcap (*Scutellaria lateriflora* L.)

- Aerial parts used in modern western herbalism as a nervine, sedative, anxiolytic
- First described in Tournefort’s *The Compleat Herbal* (1719)
- Given current name by Linneaus in 1753
- Native American medicinal use: eyewash, fever, emmenagogue, digestif, laxative, + Cherokee, Miwao, Mendocino, Iriquois, Delaware, Ojibwa (Moerman 1998)
Toxicity & Adulteration

- 1980’s reports of hepatotoxicity associated with herbal products containing skullcap
- Suspected adulteration of skullcap supply with Teucrium spp. (germander)
- Germander known toxic – attempts to prohibit sale in 90’s
- Adulteration persists...
Known Adulterant Species

- Scutellaria lateriflora
- S. alpinia L.
- S. baicalensis Georg
- S. galericulata L.
- S. incana Biehler
- S. ovata Hill
- Teucrium canadense L.
- T. chamaedrys L.
Adulteration of Skullcap with American Germander

By Steven Foster

Background

Skullcap (Scutellaria baicalensis) is a native American plant, used by Native Americans as an herbal remedy. It is also used in traditional Chinese medicine. The root of the plant is the most commonly used part in herbal preparations. Skullcap is a safe and effective herbal medicine, but it can be adulterated with other species, including American germander (Teucrium canadense), which is a widely used substitute. Adulteration can result in the loss of the therapeutic effect of the product. Several studies have evaluated the adulteration of Skullcap with American germander.

Recent Developments

The American Herbal Pharmacopoeia (AHP) has developed a protocol for testing Skullcap for the presence of adulterants. The protocol includes the analysis of specific markers present in the plant, such as flavonoids and iridoids. This protocol is designed to ensure the quality and safety of Skullcap products and to protect consumers from adulteration.

Keywords: Skullcap, adulteration, Scutellaria baicalensis, Teucrium canadense, iridoids, flavonoids.

1. Purpose

Skullcap (Scutellaria baicalensis, family Lamiaceae) herb has a long history of adulteration, evidenced in comments from over 100 years ago by Pfeiffer and Lüüs that "Scutellaria or valigna Norrell and Scutellaria arsenics Norrell are the species generally collected by herbalists and substituted for Scutellaria baicalensis." Besides the substitutions with other species from the genus Scutellaria, adulteration with germander (Teucrium) species containing iridoidic furano neo-chromenes has been reported in the early 1990s and seems to persist in the herb trade in North America and possibly elsewhere. This Laboratory Guidance Document presents a review of the various publicly-available analytical technologies and methods used to differentiate between authentic S. baicalensis and its potentially adulterating species, listed in Table 1.
on Adulteration of Skullcap

By Stefan Gföner, PhD’ and Mark Blumenthal
*Corresponding author: email

Keywords: Scutellaria lateriflora, skullcap herb, adulterant, adulteration.

Goal: The goal of this bulletin is to provide timely information and updates on issues of adulteration of Scutellaria lateriflora to the international herbal products industry and extended natural products community in general. It is intended to complement the previously published works regarding skullcap adulteration, e.g., the American Herbal Pharmacopoeia Skullcap Monograph published by Upton et al. and the article by Foster in HerbalGram by presenting new data on the occurrence of adulteration, the market situation, and consequences for the consumer and the industry.

1 General Information
1.1 Common name: Skullcap

Figures from AHP monograph 2009 (Upton et al.)
Figures from AHP monograph 2009 (Upton et al.); based on the work of Gafner et al. 2003

**Figure 11** Major constituents of *Taurum canadense* and *Taurum chamaedrys*

Verbascone: $R = H$ (*T. canadense*)

Tauroside: $R = O$ (*T. chamaedrys*)

**Table 8 Chemical differentiation between Scutellaria and Taurum species (%).**

<table>
<thead>
<tr>
<th>Species</th>
<th>Compound</th>
<th>S. interfor</th>
<th>S. baicae-</th>
<th>S. baicae-</th>
<th>S. galiroc-</th>
<th>S. inzae</th>
<th>T. canadense</th>
<th>T. chamaedrys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gajetinid</td>
<td>Gajetinid</td>
<td>0.059</td>
<td>0.057</td>
<td>0.917</td>
<td>0.048</td>
<td>0.019</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gajetinid</td>
<td>Gajetinid</td>
<td>0.039</td>
<td>0.039</td>
<td>0.001</td>
<td>4.292</td>
<td>0.022</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gajetinid</td>
<td>Gajetinid</td>
<td>0.012</td>
<td>0.024</td>
<td>0.111</td>
<td>0.148</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Scutellarin</td>
<td>Scutellarin</td>
<td>0.250</td>
<td>0.141</td>
<td>0.095</td>
<td>0.322</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Wogonin</td>
<td>Wogonin</td>
<td>0.018</td>
<td>0</td>
<td>0.949</td>
<td>0.017</td>
<td>0.014</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Teicrinoside</td>
<td>Teicrinoside</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Verbascone</td>
<td>Verbascone</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Figures 12a** HPTLC chromatogram of *Scutellaria lateriflora* and its adulterants (*Scutellaria spp. and Taurum spp.*) (UV 254)

12a) The standards scutellarin (Lane 1, R = 0.29), baicalin (Lane 1, R = 0.43), baicaolin (Lane 1, very broad zone, $R = 0.70$), and chrysin (Lane 17, R = 0.90) show dark bands. The standards teicrinoside and verbascoside (both on Lane 17) don’t show. There are bands corresponding to the standard baicaolin in all Scutellaria samples. This zone is particularly strong in 1. *S. lateriflora* (Lane 3), *S. galirocultata* (Lane 9), *S. vicicida* (Lane 10), and *S. baicaelenssis* (Lanes 11-16). The zone is fainter in the *S. lateriflora* samples on Lanes 2 and 4 and very weak in *S. barbata* on Lane 8. Scutellarin is difficult to detect in the samples, as it overlaps with baicaolin. The very broad band of baicaelin is well detected in *S. baicaelenssis* (Lanes 11-16), very strong in the root peelings on Lane 11. A dark zone is seen in many samples around the position of chrysin.

12b) The dark zones of the standards scutellarin (Lane 1, R = 0.29), baicalin (Lane 1, R = 0.24), and baicaolin (Lane 1, very broad zone, $R = 0.70$) are difficult to detect. The standards teicrinoside (Lane 14, $R = 0.90$) show light blue bands. Chrysin (Lane 17, $R = 0.90$) shows a yellowish band. There are very dark bands of various intensities in most of the Scutellaria samples corresponding to the standard baicaolin. Scutellarin is difficult to detect in the samples, as the very thick band of baicalin overlaps it. Baicaelin is only well detected in the root peelings on Lane 11. Many samples show a band corresponding to verbascoside. The strongest zone is seen in *T. canadense* (Lane 9). Strong zones are seen in *S. lateriflora* (Lane 2), *S. galirocultata* (Lane 9), *S. barbata* (Lane 6), *S. lucidum* (Lane 7), and *S. baicaelenssis* (Lanes 12, 14, and 16). Faktir

Figures AHP monograph 2009 (Upton et al.); based on the work of Gafner et al. 2003
Comparison of the Chemical Composition of Extracts from *Scutellaria lateriflora* Using Accelerated Solvent Extraction and Supercritical Fluid Extraction versus Standard Hot Water or 70% Ethanol Extraction

Chantal Bergeron,†* Stefan Gafner,† Edgar Clausen,† and Danielle J. Carrier†
Tom's of Maine, P.O. Box 710, Kennebunk, Maine 04043, 3302 Bell Engineering, University of Arkansas, Fayetteville, Arkansas 72701, and Biological and Agricultural Engineering, 203 Engineering Hall, University of Arkansas, Fayetteville, Arkansas 72701
DOI: 10.1021/jf048408t
Publication Date (Web): March 18, 2005
Copyright © 2005 American Chemical Society

Research Article

Comparison of the Phenolic Component Profiles of Skullcap (*Scutellaria lateriflora*) and Germander (*Teucrium canadense* and *T. chamaedrys*), a Potentially Hepatotoxic Adulterant

Long-Ze Lin,* James M. Harnly* and Roy Upton*

ABSTRACT:
Introduction – *Scutellaria lateriflora*, commonly known as skullcap, is used as an ingredient in numerous herbal products. Unfortunately, it has occasionally been adulterated with *Teucrium canadense* or *T. chamaedrys*, commonly known as germander, which contains potentially hepatotoxic diterpenes. Chromatographic profiles of the phenolic components provide a means of distinguishing between these plants and enhancing public safety.

Objective – To develop a chromatographic method for the identification of *Scutellaria lateriflora* and two *Teucrium* species and to quantify the latter as adulterants.

Methodology – Samples were extracted with aqueous methanol and the extracts were analysed using a standardised LC-DAD-ESI/MS profiling method to obtain their phenolic profiles.

Results – Skullcap contained primarily flavonoids, while the major phenolic components of the two *Teucrium* species were the phenylethanoids, verbascoside and teucroside. Using the phenylethanoids as markers, it was possible to clearly distinguish between the two genera and to determine 5% *Teucrium* mixed with *Scutellaria* using either ultraviolet absorption spectrometry...
### Table 3. Comparison among the different techniques to authenticate S. lateriflora

<table>
<thead>
<tr>
<th>Method</th>
<th>Applicable to</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroscopic</td>
<td>Raw material</td>
<td>Quick preparation, no solvents required</td>
<td>No automation/statistics, outcome relies on analyst's expertise, difficult or impossible for cv material</td>
</tr>
<tr>
<td>Microscopic</td>
<td>Raw material</td>
<td>Quick preparation, easily detect adulterating non-target species</td>
<td>No automation/statistics, outcome relies on analyst's expertise, difficult or impossible for cv material</td>
</tr>
<tr>
<td>Genetic</td>
<td>Raw material</td>
<td>Able to distinguish closely related species, detect small amounts of adulterants</td>
<td>Labor-intensive sample preparation and analysis, expensive equipment, cannot distinguish among plant parts</td>
</tr>
<tr>
<td>HPLC</td>
<td>Raw material, extracts</td>
<td>Quick method systems affordable for smaller labs, detect small amounts of adulterants</td>
<td>No statistics, high-end equipment expensive, need for standard compound prepares</td>
</tr>
<tr>
<td>HPLC-UV</td>
<td>Raw material, extracts</td>
<td>Equipment in many laboratories, detect small amounts of adulterants, mostly quantitative less specific than HPLC-UV/MS</td>
<td>Equipment expensive, often no statistics applied, although software is available, need for standard compound prepares</td>
</tr>
<tr>
<td>HPLC-UV/MS</td>
<td>Raw material, extracts</td>
<td>Standard equipment in many laboratories, detect small amounts of adulterants, qualitative and quantitative</td>
<td>Equipment very expensive, initial setup of parameters complex, quality of data depends on ability to refine analysis, need for standard compound prepares</td>
</tr>
<tr>
<td>Standard MS (flow injection MS)</td>
<td>Raw material, extracts</td>
<td>Short analysis time, reliable and highly reproducible, qualitative and quantitative</td>
<td>Equipment very expensive, initial setup of parameters complex, quality of data depends on ability to refine analysis, needs at least 4 x 7 floor space</td>
</tr>
<tr>
<td>NMR</td>
<td>Raw material, extracts</td>
<td>Short analysis time, reliable and highly reproducible, qualitative and quantitative</td>
<td>Equipment and maintenance very expensive, initial setup of parameters complex, labor- and time-intensive sample preparation, needs at least 4 x 7 floor space</td>
</tr>
</tbody>
</table>

### Table 4. Comparison among different published HPLC methods for S. lateriflora

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of samples (aerial parts when not specified)</th>
<th>Sample preparation: handling / duration (mins)</th>
<th>Column type</th>
<th>Run time (mins)</th>
<th>Detection wavelength (UV) or ion mode (MS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[16]</td>
<td>1 Commercial raw material</td>
<td>15 / 200</td>
<td>C18</td>
<td>75</td>
<td>MS (negative)</td>
</tr>
<tr>
<td>[17]</td>
<td>9 AHP</td>
<td>5 / 90</td>
<td>C18</td>
<td>75</td>
<td>UV 280, 310, 330, 350 MS (pos/neg)</td>
</tr>
<tr>
<td>[18]</td>
<td>10 Commercial products</td>
<td>ASE1</td>
<td>C18</td>
<td>85</td>
<td>UV 278</td>
</tr>
<tr>
<td>17</td>
<td>AHP &amp; internet</td>
<td>7 / 95</td>
<td>C18</td>
<td>18</td>
<td>MS (negative)</td>
</tr>
<tr>
<td>1</td>
<td>Grown from seeds</td>
<td>8 / 150</td>
<td>C18</td>
<td>36</td>
<td>UV 280</td>
</tr>
<tr>
<td>7</td>
<td>Commercial products off shelf</td>
<td>3h / 15h</td>
<td>C18</td>
<td>32</td>
<td>UV 270</td>
</tr>
<tr>
<td>8</td>
<td>Research Center for Medicinal Plant Resources, Tsukuba &amp; commercial sources</td>
<td>15 / 180</td>
<td>C18</td>
<td>24</td>
<td>UV 277</td>
</tr>
<tr>
<td>1</td>
<td>Grown from seeds</td>
<td>ASE1</td>
<td>C18</td>
<td>33</td>
<td>UV 270</td>
</tr>
<tr>
<td>1</td>
<td>Tissue culture from seeds</td>
<td>10 / 183</td>
<td>C18</td>
<td>60</td>
<td>MS (positive)</td>
</tr>
<tr>
<td>2</td>
<td>AHP &amp; commercial raw material</td>
<td>7 / 170</td>
<td>C18</td>
<td>30</td>
<td>UV 280</td>
</tr>
</tbody>
</table>
SMPr Key Points: Skullcap

3 SMPrs:
• Quantification of Flavonoids
• Limit Test
• Identity

Method Performance Requirements:
Determination of Select Flavonoids From Skullcap

Table 3: Analytical Range & LOQ

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical range (%, w/w)</td>
<td>1 - 50</td>
</tr>
<tr>
<td>Limit of Quantitation (%, w/w)</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 4: Method Performance Requirements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Recovery</td>
<td>93 - 105</td>
</tr>
<tr>
<td>% RSD&lt;sub&gt;r&lt;/sub&gt;</td>
<td>≤ 5</td>
</tr>
<tr>
<td>% RSD&lt;sub&gt;b&lt;/sub&gt;</td>
<td>≤ 8</td>
</tr>
</tbody>
</table>
**Method Performance Requirements:**
Limit Test for Determination of Selected Compounds from *Teucrium* spp. in Skullcap Materials in Commerce

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD</td>
<td>10 ppm</td>
</tr>
</tbody>
</table>

**Table 3: LOD**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum Requirement (at detection limit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Recovery</td>
<td>80 - 110</td>
</tr>
<tr>
<td>RSD_R</td>
<td>7</td>
</tr>
<tr>
<td>RSD_2</td>
<td>11</td>
</tr>
</tbody>
</table>

**Method Performance Requirements:**
Identification of Skullcap in Raw Materials, Skullcap-based Dietary Ingredients, and Dietary Supplements

**Table 1: Method Performance Requirements**

| Selectivity Study | 90% probability of identification with 95% confidence (33 correct identifications out of 33 samples known to contain skullcap). *See Validation Guidance in section II of this document.* |

*Some aberrations may be acceptable if the aberrations are investigated, and acceptable explanations can be determined and communicated to method users.*
## SMPIRs Comments & Responses

<table>
<thead>
<tr>
<th>Type of Comment</th>
<th>Comment or Concern (Justification for Change - Include Line Number if Applicable)</th>
<th>Proposed Change(s) - Please include line number(s)</th>
<th>CSO Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technical</td>
<td>Analytical range doesn’t define range.</td>
<td>Include full definition.</td>
<td>There is in fact a definition for “analytical range” in the SMPR that is more like a description than a definition. Ask commentor to suggest a definition.</td>
</tr>
</tbody>
</table>

## Motion to Approve Resveratrol SMPIR

**Move to approve the Standard Method Performance Requirements (SMPIRs) for:**

- **Determination of Select Flavanoids from Skullcap**
- **Identification of Skullcap in Raw Materials, Skullcap-based Dietary Ingredients, and Dietary Supplements**
- **Limit Test for Determination of Selected Compounds from Teucrium spp. In Skullcap Materials in Commerce**
Discussion?