AOAC INTERNATIONAL Presents…

the Stakeholder Panel on Dietary Supplements

(SPDS)

FRIDAY, MARCH 17, 2017, 8:30 a.m.
Salon C/D/E

MARRIOTT WASHINGTONIAN CENTER
9751 WASHINGTONIAN BOULEVARD
GAITHERSBURG, MARYLAND
UNITED STATES

contact: spds@aoac.org

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STAKEHOLDER PANEL CHAIRS

DARRYL SULLIVAN, COVANCE LABORATORIES
Chair, AOAC Stakeholder Panel on Dietary Supplements

Darryl Sullivan is a Fellow of AOAC and has been an active member since 1980. He has served terms as secretary, president-elect, president, past president, and director of the Board of Directors, and previously served a three-year term as chair of the Official Methods Board, and is currently serving as Chair of the AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals. In 2012 Darryl lead a very successful AOAC engagement with government and industry thought leaders in India and China on behalf of SPIFAN. He is also active with the Stakeholder Panel for Strategic Food Analytical Methods and the Stakeholder Panel for Agent Detection Assays. Sullivan also served a three-year term as a director on the AOAC Research Institute Board of Directors. He was a founding member and chair of the Presidential Task Force on Dietary Supplements and a member of the Task Force on Bacillus anthracis, as well as the AOAC Task Force on Nutrition Labeling and the AOAC Task Force on Sulfites. Prior to chairing the OMB, he served as a member and chair of the Methods Committee on Commodity Foods and Commodity Products. Sullivan was a founding member of the AOAC Technical Division on Reference Materials and served three terms on the Division's Executive Board. A staunch supporter of the Association, Sullivan was active in the e-CAM and Scholar I projects at AOAC, has exhibited at the annual meetings for many years, has presented hundreds of papers and posters at AOAC meetings and regularly publishes his research in the journal of the AOAC. He has also presented a significant number of papers on behalf of AOAC at other scientific meetings in many different parts of the world.

BRIAN SCHANEBERG, STARBUCKS COFFEE CO.
Vice Chair, AOAC Stakeholder Panel on Dietary Supplements

Brian Schaneberg, Ph.D., is the Global Scientific & Regulatory Affairs Director for Starbucks Coffee Company. Brian participates in the execution of company strategies while ensuring compliance and regulatory guidelines are met and followed by the company across all products: Starbucks, Teavana, Tazo, Evolution Fresh, La Boulange, and Ethos. Brian has over 15 years of natural products experience in the area of dietary supplements and herbals. Brian was also the Quality & Food Saftey and Scientific & Regulatory Affairs Director for Mars Botanical, a division of Mars, Inc. focusing on cocoa flavanol science and products. Before Mars Botanical, he was the Director of Technical Services at ChromaDex, Inc. in Irvine, California and was an Associate Research Scientist at the National Center for Natural Products Research at the University of Mississippi under the guidance of Dr. Ikhlas Khan, in a position funded by the US FDA for the development of methods to ensure the quality and safety of botanicals and dietary supplements. Over the years, Brian has worked closely with trade groups, industry, academia and government leaders. He has been a member of various review committees including NIH grants, analytical validation ERPs at AOAC and the Registry of Carcinogens. Brian also had the pleasure of holding an adjunct faculty position at the University of Colorado, Denver, advising a student that received his MS in Analytical Chemistry isolating phytochemicals and developing analytical testing procedures for Horse Chestnut. Brian has a Ph.D. in Organic Chemistry from Virginia Commonwealth University and a B.A. in Chemistry with a minor in Biology from Central College in Iowa. He has authored or co-authored more than 50 publications and presentations.

PRESENTER BIOS

ANTON BZHELYANSKY, USP
CHAIR, GINGER WORKING GROUP

Anton Bzhelyansky holds a Master's degree in analytical chemistry from the University of Maryland Baltimore County. His thesis, under the direction of George M. Murray, was on uranyl-templated polymers. Upon graduation, he spent 13 years working for generic pharmaceutical and dietary supplement companies, primarily as a method developer. Anton's analytical portfolio includes methodologies for a broad spectrum of analytes, from conventional pharmaceutical APIs to complex dietary supplement formulations, from marine oils to vitamins, chondroitins and botanicals. During his tenure in the dietary supplement industry, he implemented total inspection of incoming raw materials by NIR, established ICP-OES routine analysis, studied sampling of incoming ingredients and in-process blends, worked on formulation of enteric-coated dosage forms, and served as a Waters Empower® administrator. An AOCS Approved Chemist in 2011-2012, Anton developed a 20-minute marine oil GC method (poster at AOAC 125th Annual Meeting) and optimized Peroxide and Anisidine Value analyses. His most memorable analytical work, however, remains the suite of methods for monitoring glucosinolates and isothiocyanates in formulations involving *Cruciferae*, including assessment of their enzymatic conversion rate. Anton has been with USP for four. He is responsible for the majority of botanical monographs in the *USP-NF* Dietary Supplements section. Anton dedicated a significant effort to development of the USP General Chapter <2251> *Screening For Undeclared Drugs and Drug Analogues,* and is currently compiling the USP Adulterants database. In line with the USP's "Up-To-Date" policy, he is continuously working to improve compendial analyses. Anton is interested in implementing advanced techniques for challenging analytes such as oligomeric proanthocyanidins and complex polysaccharides, as well as devising a practical route for adoption of chemometric procedures in pharmacopeial monographs. He is a member of AOAC (2004) and AOCS (2008).

KAN HE, HERBALIFE
SPDS ALOE VERA WORKING GROUP

Kan He is responsible for development of botanical ingredients for Herbalife product line. He has been involved in botanical product design and development from lab scale to commercial production.

Before joined Herbalife, Kan He was in charge of research and development at Pure World Botanicals, Inc. and Naturex, Inc. respectively. He was responsible for developing new products and new processes, including scale up of plant extraction, purification, and chemical characterization of standardized herbal extracts.

Kan He graduated from the Shanghai University of Traditional Chinese Medicine with BSc and MSc in Pharmacy and Medicinal Chemistry. He received his Ph.D. in pharmacognosy from the Pharmaceutical Sciences, University of Arizona and completed his postdoctoral research at School of Pharmacy, Purdue University. Over the past twenty-

five years, he has been working in the area of natural products chemistry and authored or co-authored over 70 research papers on the peer reviewed scientific journals and book chapters. Kan He holds 11 US patents on the development of new herbal ingredients and new herbal manufacturing processes.

INGER REIDUN AUKRUST, KAPPA BIOSCIENCE
SPDS Vitamins K_1 and K_2 Working Group

Inger Reidun Aukrust holds a PhD in Organic chemistry in 1995 at the University of Oslo. Established own firm Synthetica in 2000. Synthetica is an CRO in synthetic chemistry within pharma. Established Kappa Bioscience in 2006. Kappa Bioscience is Vitamin K2 MK7 manufacturer and supplier.

JOSEPH ZHOU, SUNSHINEVILLE HEALTH PRODUCTS
SPDS SAMe Working Group

Dr. Joseph Zhou has been working in the dietary supplement industry since 1996. He is currently the technical director of Sunshineville Health Products, Inc, in charge of both products development and analytical methods development. He was also a technical director in a few of other famous brands companies in the US. He has been actively participating in the AOAC official methods program since 2002. His team established the AOAC official method of Glucosamine. He was one of the important players in the AOAC single lab validation projects for Chondroitin Sulfates and MSM, and was involved in many other AOAC methods projects. Dr. Zhou is the author of the USP monograph of Arginine. He is an adjunct professor of pharmacognosy at College of Pharmacy, University of Illinois at Chicago. He was awarded by AOAC as the Study Director of the Year of 2005.

GARRETT ZIELINSKI, COVANCE
SPDS FREE AMINO ACIDS WORKING GROUP

Garrett Zielinski is a Program Development Manager at Covance Laboratories in Madison, WI. Mr. Zielinski acts as the primary liaison for dietary supplement clients as well as providing expertise on designing and managing testing programs to meet scientific and regulatory requirements. He also acts as a technical resource for customers as needed for analytical troubleshooting. He has designed and managed raw material, in-process, finished product, stability, and retail audit testing programs. He participates in a number of organizations involved with the dietary supplement industry related to regulation and analytical testing.

Mr. Zielinski has over 13 years of experience in organic and analytical chemistry related to pharmaceuticals, foods and dietary supplements. He has authored a number of scientific posters, journal articles, and scientific presentations related to analytical testing of food and dietary supplements.

AOAC INTERNATIONAL

The Scientific Association Dedicated to Analytical Excellence®

MARCH 17, 2017

GAITHERSBURG MARRIOTT WASHINGTONIAN CENTER
9751 WASHINGTONIAN BLVD, GAITHERSBURG, MD, 20878
CONFERENCE ROOM: SALON C-D-E

8:30am – 5:00pm Eastern Standard Time
Registration Opens at 7:30am

STAKEHOLDER PANEL ON DIETARY SUPPLEMENTS (SPDS)

Chair: Darryl Sullivan, Covance
Vice Chair: Brian Schaneberg, Starbucks

A G E N D A

I. **Welcome and Introductions (8:30-8:40am)**
Jonathan Goodwin, AOAC and Darryl Sullivan, Covance (Chair, SPDS)

II. **Ingredient Updates (8:40am – 9:00am)**
Darryl Sullivan

a. Status of Ingredients to Date

b. Open Calls for Methods and Calls for Experts (Protein, Vitamin B12 + Open Calls for Cinnamon, Collagen, Folin C and Kratom)

III. **SMPR Presentations and Consensus* (9:00am – 12:30pm)**

a. Vitamin D (9:00 am – 9:15pm)
Chair: John Austad, Covance, Chair of the Vitamin D Working Group

b. Aloe Vera (9:15am – 10:00am)
Chair: Kan He, Herbalife, Chair of the Aloe Vera Working Group

c. Ginger (10:15am – 11:00am)
Chair: Anton Bzhelyansky, USP, Chair of the Ginger Working Group

d. Free Amino Acids (11:00am – 11:45am)
Chair: Garrett Zielinski, Covance, Chair of the FAA Working Group

e. Vitamins K1 and K2 (11:45am – 12:30pm)
Chair: Inger Reidun Aukrust, Kappa Biosciences, Chair of the Vitamin K Working Group

IV. **SPDS Advisory Panel Update (1:30pm – 1:45pm)**

a. December Advisory Panel Meeting & Future Priorities
Darryl Sullivan

V. **Launch of Set 7 Working Groups (1:45pm – 4:30pm)**

a. Working Group Launch Presentation: Echinacea (1:45pm – 2:45pm)
Chair: Stefan Gafner, American Botanical Council

b. Working Group Launch Presentation: Ginseng (3:00pm – 4:00pm)
Chair: Paula Brown, British Columbia Institute of Technology

c. Working Group Launch Presentation: SAMe (4:00pm – 5:00pm)
Chair: Joseph Zhou, Sunshineville Health Products

VI. **Adjourn**

Morning Break: 10:00am – 10:15am | Lunch (on your own): 12:30pm – 1:30pm | Afternoon Break 2:45pm – 3:00pm

**Item(s) requires a vote by SPDS*

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 Methods*SM for the prioritized dietary supplement ingredients
- Encourage participation with the dietary supplements industry to develop voluntary consensus standards.

Stakeholder Panel on Dietary Supplements (SPDS)

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

Stakeholder Panel on Dietary Supplements (SPDS)

- **Set 2 Ingredients: Ashwagandha, Cinnamon, Folin C and Kratom**
  - Launched September, 2014
  - SMPRs Approved in March, 2015:
    - Withanolide Glycosides and Aglycones of Ashwagandha (2015.007)
    - Alkaloids of Mitragyna speciosa (Kratom) (2015.008)
    - Estimation of Total Phenolic Content Using the Folin-C Assay (2015.009)
    - Identification of Selected Cinnamomum spp. Bark in Dietary Supplement Raw Materials and/or Finished Products (2015.010)
- First Action OMA for One (1) Ashwagandha Method
- Call for Methods and Experts currently posted for Kratom and Folin-C. Deadline is March 31, 2017. 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

Stakeholder Panel on Dietary Supplements (SPDS)

- **Set 4 Ingredients: Collagen, Lutein, Turmeric**
  - Launched in September, 2015
  - SMPRS Approved in March, 2016:
    - Quantitation of Curcuminoids (2016.003)
    - Quantitative Measurement of β-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 B_{12}**
  - Launched in March, 2016
  - SMPRs Approved in September, 2016:
    - Identification of Proteins in Dietary Supplements
      - Animal Derived (2016.015) and Non-Animal Derived (2016.016)
    - Identification and Quantitation of Proteins in Dietary Supplements
      - Animal Derived (2016.013) and Non-Animal Derived (2016.014)
    - Quantitative Measurement of Vitamin B_{12} 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 K_1 and K_2
  - 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

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

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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

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_2 (ergocalciferol), vitamin D_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 De initions

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 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_r); or % repeatability relative standard deviation (%RSD_r).

Reproducibility.—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.—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.

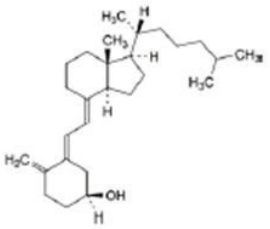
5 System Suitability Tests and/or Analytical Quality Control

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.

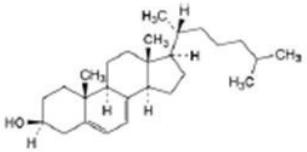
6 Reference Material(s)

NIST Standard Reference Material® 3280; the reference value of vitamin D_2 in NIST 3280 is 8.6 µg/g (±2.6) µg/g vitamin D_2.

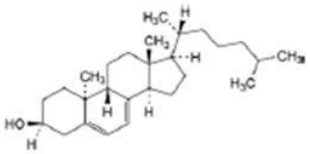
NIST Standard Reference Material® 3532 D_3; the reference value of vitamin D_3 in NIST 3532 is 1.310 ± 0.033 µg/g cholecalciferol (vitamin D_3).



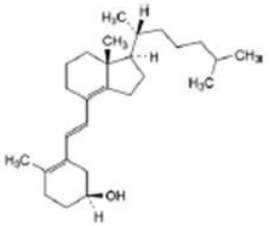
A. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-trien-3β-ol (*trans*-cholecalciferol, *trans*-vitamin D_3),



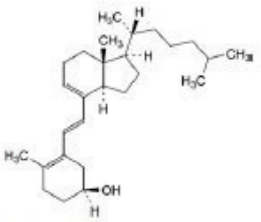
B. cholesta-5,7-dien-3β-ol (7,8-didehydrocholesterol, provitamin D_3),



C. 9β,10α-cholesta-5,7-dien-3β-ol (lumisterol$_3$),



D. (6*E*)-9,10-secocholesta-5(10),6,8(14)-trien-3β-ol (iso-tachysterol$_3$),



E. (6*E*)-9,10-secocholesta-5(10),6,8-trien-3β-ol (tachysterol$_3$).

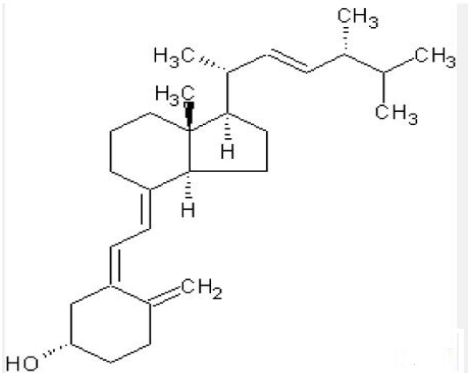


Figure 1. Chemical structure of vitamin D_2 (ergocalciferol), vitamin D_3 (cholecalciferol), and their previtamin D and hydroxy forms.

7 Validation Guidance

Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis, *Official Methods of Analysis* (current edition), AOAC INTERNATIONAL, Rockville, MD, USA. Available at: http://www.eoma.aoac.org/app_d.pdf

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.

Approved by AOAC Stakeholder Panel on Dietary Supplements (SPDS). Final Version Date: September 25, 2015. Effective Date: September 25, 2015.

Table 1. Analytical range and LOQ based on matrix

Parameter	Finished products	Ingredients
Analytical range ppm[a]	0.5–12 500	1250–12 500
Limit of quantitation ppm[a]	0.4	1000

[a] Measured as individual forms of vitamin D and pre-vitamin D.

Table 2. Method performance requirements as a function of range

	Range, µg/g[a]				
Parameter	<10–15	>15–50	>50–500	>500–4000	>4000–12 500
Recovery, %	80–110	90–107	95–105	95–105	97–103
Repeatability (RSD_r), %	8	7	5	4	3
Reproducibility (RSD_R), %	12	10	8	6	4

[a] Measured as individual forms of vitamin D and pre-vitamin D.

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

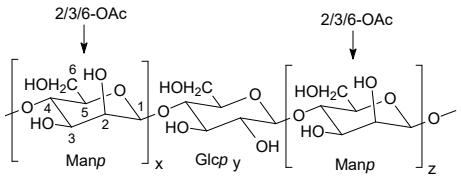
Aloe Vera Working Group Work to Date

- 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

Background

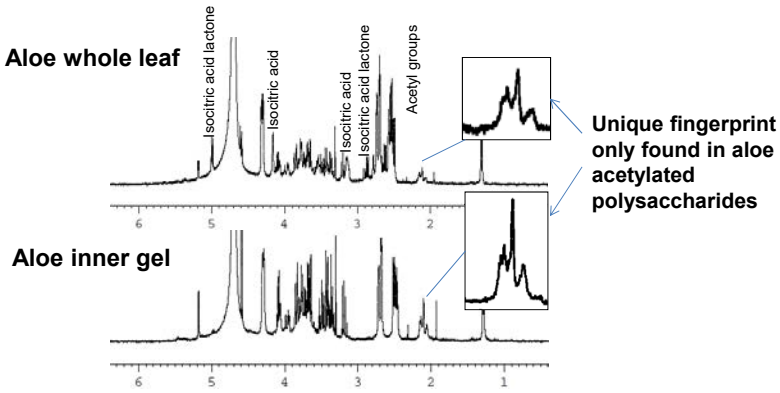
Summary of current methods used in Aloe qualification (identification) and quantitation analysis:

- ^{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;

Background

Summary of current methods used in Aloe qualification (identification) and quantitation analysis (*cont'd*):

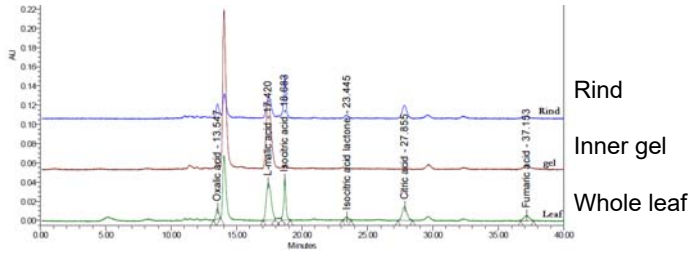
- Example of aloe identification by ^{1}H NMR



Background

Summary of current methods used in Aloe qualification (identification) and quantitation analysis (*cont'd*):

- 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;

Background

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;

Background

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

Parameter	Ingredients (Raw Materials)	Finished Products - Solid	Finished Products – Liquid (Samples to be freeze dried before analysis)
LOQ (%)	≤ 0.5	≤ 0.5	≤ 0.15
Analytical Range (%)	1 – 100	1 – 100	0.15 – 100

Recovery, Repeatability & Reproducibility

Parameter	Ingredients (Raw Materials) (1 – 100%)	Finished Products – Solid (1 – 100%)	Finished Products – Liquid (Samples to be freeze dried before analysis)	
			0.15 – 0.5%	≥ 0.5 – 100%
Recovery (%)	90 – 110	90 – 110	≥ 50	90 – 110
% RSD_r	≤ 10	≤ 10	≤ 20	≤ 10
% RSD_R	≤ 15	≤ 15	≤ 30	≤ 15

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?

DRAFT AOAC SPDS Aloe Vera SMPR, v6, March 10, 2017.

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-ß-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

Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements*, 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

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 D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_d.pdf

Appendix F: Guidelines for Standard Method Performance Requirements; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

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

Liquid
Powder
concentrates
purified polysaccharides
processed polysaccharides

Table 2: Dietary Supplements

Tablets
Capsules
Liquids
Powders
Extracts
Gummies
Softgels

Table 3: Potential Adulterants

Maltodextrin
Carragennan
Gum acacia
Locust gum

Table 4: Method performance requirements.

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.	

DRAFT AOAC SPDS Aloe Vera SMPR, v6, 16 November 2016.

Quantitation of Aloe Vera Polysaccharides in Dietary Supplements

Intended Use: Reference method for cGMP compliance.

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

2. **Applicability**:
Quantitation of total 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 (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 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)

Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements*, 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

8. Validation Guidance:

Data demonstrating that the candidate method meets the performance criteria should be submitted for the adulterants listed in Table 3 and the matrices listed in Table 4.

Pharmachem Labs may provide materials for evaluation.

Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_d.pdf

Appendix F: Guidelines for Standard Method Performance Requirements; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

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).

Parameter	Ingredients (Raw Materials)	Finished Products - Solid	Finished Products – Liquid (Freeze dried samples)
LOQ (%)	≤ 0.5	≤ 0.5	≤ 0.15
Analytical Range (%)	1 – 100	1 – 100	0.15 – 100

Table 2: Method performance requirements (part 2).

Parameter	Ingredients (Raw Materials) (1 – 100%)	Finished Products – Solid (1 – 100%)	Finished Products – Liquid (Freeze dried samples)	
			0.15 – 0.5%	≥ 0.5 – 100%
Recovery (%)	90 – 110	90 – 110	≥ 50	90 – 110
% RSD_r	≤ 10	≤ 10	≤ 20	≤ 10
% RSD_R	≤ 15	≤ 15	≤ 30	≤ 15

Table 3: Potential Adulterants

Maltodextrin
Carageenan
Gum acacia
Locust gum

Table 4 : List of Matrices

Tablets
Capsules
Liquids
Powders
Extracts
Plant products

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 Szpylka, 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

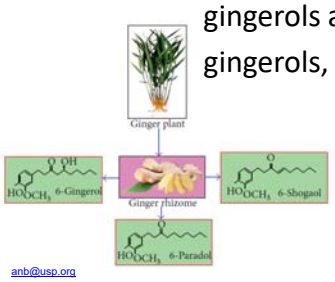
- **In-Person Launch Meeting** (September 16, 2016 at the AOAC Annual Meeting, Dallas, TX)
- **2 Teleconferences** (October 27 & November 10, 2016)
- **1 SMPR** Drafted: ***Quantitation of Select Nonvolatile Ginger Constituents***
- Public comment period: December 23, 2016 – January 27, 2017. No public comments received.
- 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

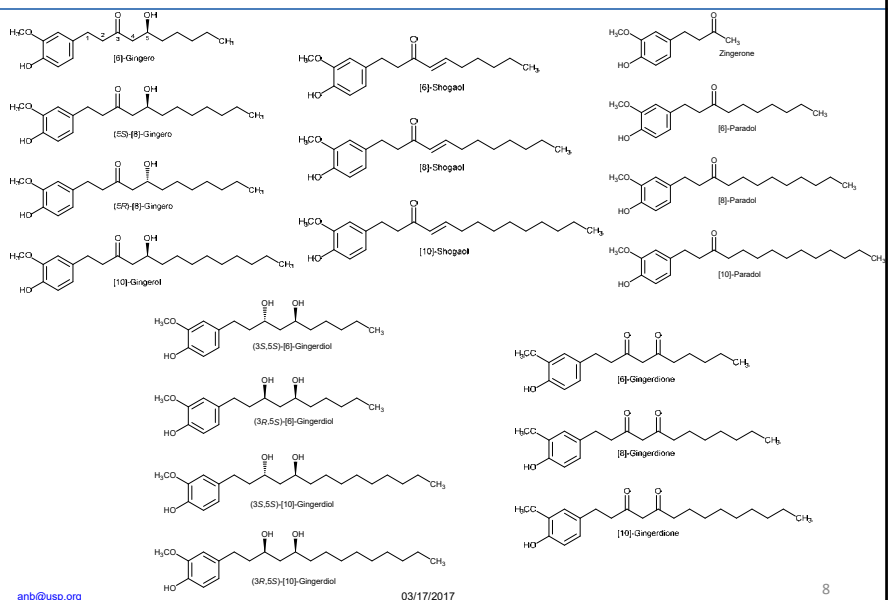
Background

- 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									
	Gingerols			Shogaols			Paradols			Zingerone
	[6]-	[8]-	[10]-	[6]-	[8]-	[10]-	[6]-	[8]-	[10]-	
Chengdu Biopurify	X	X	X	X	X	X				X
Chromadex	X	X	X	X	X	X				
Extrasynthese	X	X		X						
Phytolab	X	X	X	X	X	X				
Sigma-Aldrich	X	X	X	X		X				X
Tokiwa	X	X	X	X						
Dalton Research	X	X	X	X			X	X	X	

Ginger Analytes with Chemical Identifiers

Constituent	IUPAC Name	Formula	CAS Number	UNII Code	InChi Key	PubChem
[6]-Gingerol	(S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one	C17H26O4	23513-14-6	92SQKZZ900	NLDDIKRKFXEWBK-AWEZNQCLSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/442793
[8]-Gingerol (S)	(S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one	C19H30O4	23513-08-8	L80UB138K	BCIWKKMTBRYQJU-INIZCTEOSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/168114
[8]-Gingerol (R)	(R)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one	C19H30O4	135272-33-2	---	BCIWKKMTBRYQJU-MRXNPFEDSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/11023711
[10]-Gingerol	(S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)tetradecan-3-one	C21H34O4	23513-15-7	ND6ZLI4JOV	AIULWNKTYPZYAN-SFHVURJKSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/168115
[6]-Shogaol	(E)-1-(4-hydroxy-3-methoxyphenyl)dec-4-en-3-one	C17H24O3	555-66-8	83DNB5FIRF	OQWKEEOHDMUXEO-BQYQJAHWSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/5281794
[8]-Shogaol	(E)-1-(4-hydroxy-3-methoxyphenyl)dodec-4-en-3-one	C19H28O3	36700-45-5	AV4IK2HCNT	LGZSMXJRMTYABD-MDZDMXLPSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/6442560
[10]-Shogaol	(E)-1-(4-hydroxy-3-methoxyphenyl)tetradec-4-en-3-one	C21H32O3	36752-54-2	UP39BHE708	FADFGCOCHHNRHF-VAWYXSNFSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/6442612
[6]-Gingerdiol (3R,5S)	(+)-(3R,5S)-1-(4-hydroxy-3-methoxyphenyl)decane-3,5-diol	C17H28O4	154905-69-8	4C9F8U79BX	QYXKQNMJTHPKBP-LSDHHAIUSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/11369949
[6]-Gingerdiol (3S,5R)	(-)-(3S,5R)-1-(4-hydroxy-3-methoxyphenyl)decane-3,5-diol	C17H28O4	53318-09-5	4C9F8U79BX	QYXKQNMJTHPKBP-LSDHHAIUSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/11369949
[6]-Gingerdiol (3S,5S)	(3S,5S)-1-(4-hydroxy-3-methoxyphenyl)decane-3,5-diol	C17H28O4	143615-76-3	---	QYXKQNMJTHPKBP-GJZGRUSLSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/15839040
[8]-Gingerdiol (3S,5S)	(3S,5S)-1-(4-hydroxy-3-methoxyphenyl)dodecane-3,5-diol diacetate	C19H32O4	863780-91-0	---	BUACOWOGXVQEBF-VJOGAFQXNA-N	
[8]-Gingerdiol (3R,5S)	(3R,5S)-1-(4-hydroxy-3-methoxyphenyl)dodecane-3,5-diol	C19H32O4	53254-76-5	---	RLBBNYBPCMIQMG-DLBZATESA-N	
[10]-Gingerdiol (3S,5S)	(3S,5S)-1-(4-hydroxy-3-methoxyphenyl)tetradecane-3,5-diol	C21H36O4	1438241-35-0	---	LGSIUDXMEDKEPY-OALUTQOASA-N	https://pubchem.ncbi.nlm.nih.gov/compound/101572265
[10]-Gingerdiol (3R,5S)	(3R,5S)-1-(4-hydroxy-3-methoxyphenyl)tetradecane-3,5-diol	C21H36O4	53254-77-6	---	LGSIUDXMEDKEPY-RBUKOAKNSA-N	
[10]-Gingerdiol (3S,5R)	(3S,5R)-1-(4-hydroxy-3-methoxyphenyl)tetradecane-3,5-diol	C21H36O4	1339934-29-0	---	LGSIUDXMEDKEPY-QINVSXPYNA-N	
[6]-Gingerdione	1-(4-hydroxy-3-methoxyphenyl)decane-3,5-dione	C17H24O4	61871-71-4	L2L6JCL6YY	KMNVXQHNIWUUSE-UHFFFAOYSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/162952
[8]-Gingerdione	1-(4-hydroxy-3-methoxyphenyl)dodecane-3,5-dione	C19H28O4	77334-06-6	70E1Y63Q2L	QDSRAFNZQKMHPZ-UHFFFAOYSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/14440537
[10]-Gingerdione	1-(4-hydroxy-3-methoxyphenyl)tetradecane-3,5-dione	C21H32O4	79067-90-6	---	QPSYZJDGMPQMSV-UHFFFAOYSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/14440539
Zingerone	4-(4-hydroxy-3-methoxyphenyl)butan-2-one	C11H14O3	122-48-5	4MMW8S0B92	QJYLAHXKWMRDGS-UHFFFAOYSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/31211
6-Paradol	1-(4-hydroxy-3-methoxyphenyl)decan-3-one	C17H26O3	27113-22-0	BO24ID7E9U	CZNLTCTYLMYLHL-UHFFFAOYSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/94378
8-Paradol	1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one	C19H30O3	27113-23-1	---	TYQRTQZWHUXDLG-UHFFFAOYSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/213821
10-Paradol	1-(4-hydroxy-3-methoxyphenyl)tetradecan-3-one	C21H34O3	36700-48-8	---	XNBUKRQGYHYOOP-UHFFFAOYSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/51352076

Note: Stereoisomers presumed to be naturally prevalent are shown in yellow.

Analytical Methods (LC-UV)

JOURNAL OF AOAC INTERNATIONAL VOL. 90, NO. 5, 2007

Liquid Chromatographic Determination of 6-, 8-, 10-Gingerol, and 6-Shogaol in Ginger (*Zingiber officinale*) as the Raw Herb and Dried Aqueous Extract

SAMUELA LEE, CHEANG KHOO, CLYNTON WADE HALSTEAD, THUY HUYNH, and ALAN BENSOUSSAN

Table 2. Comparison of extraction methods

	Mean concn extracted, mg/g ± RSD, %[a]			
Method	6-Gingerol	8-Gingerol	10-Gingerol	6-Shogoal
Reflux	9.6 ± 2.0	1.3 ± 1.8	1.9 ± 1.5	1.7 ± 2.4
Ultrasonication	9.6 ± 1.6	1.3 ± 3.3	2.0 ± 3.5	1.7 ± 1.9
Soxhlet	8.0 ± 16.3	1.2 ± 12.3	1.6 ± 17.9	1.8 ± 9.2

[a] $n = 5$.

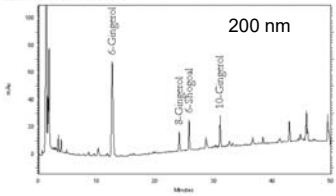


Figure 2. Chromatogram obtained by LC-PDA for the ginger raw herb (λ = 200 nm, 6-gingerol at 10.3 min, 8-gingerol at 20.4 min, 6-shogaol at 20.6 min, and 10-gingerol at 30.1 min).

Table 4. Analyte concentrations in the raw herb and dried aqueous extract

	Mean concn, mg/g ± RSD, %[a]			
Matrix	6-Gingerol	8-Gingerol	10-Gingerol	6-Shogoal
Raw herb	9.3 ± 0.8	1.6 ± 0.5	2.3 ± 1.1	2.3 ± 1.0
Dried aqueous extract	1.8 ± 2.0	0.1 ± 3.8	0.2 ± 4.1	2.9 ± 1.7

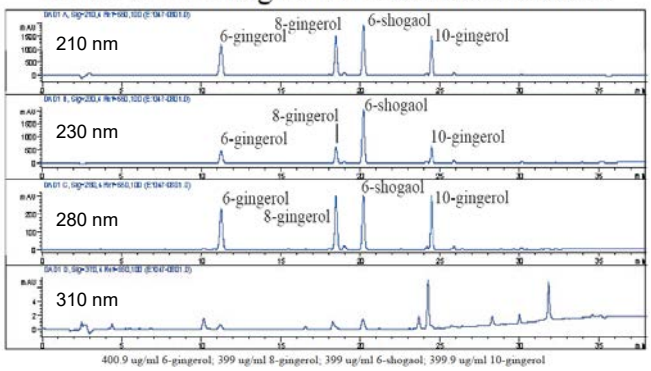
[a] $n = 7$.

Analytical Methods (LC-UV)

Extraction and Analysis of Fresh Ginger Root and Ginger Dietary Supplement

Gábor G. Angeli*, Verónica P. Rodríguez**, Barbara N. Timmermann**, Anikó M. Sólyom**
*Catalina Foothills High School, Tucson, Arizona 85718
**Arizona Center for Phytomedicine Research, University of Arizona, Tucson, AZ 85721

HPLC Chromatogram of a Standard Mixture



400.9 ug/ml 6-gingerol; 399 ug/ml 8-gingerol; 399 ug/ml 6-shogaol; 399.9 ug/ml 10-gingerol

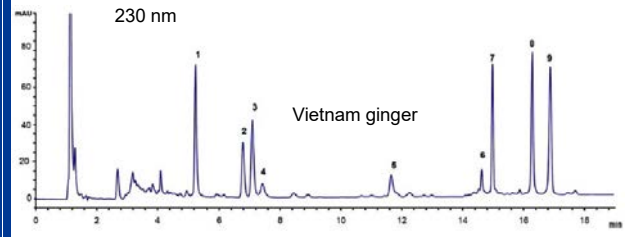
"Amongst the analyzed components 6-shogaol was found almost exclusively in the extracts of the dietary supplement ginger sample".

Analytical Methods (LC-UV)

Food Chemistry 158 (2014) 101–111

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

Soparat Yudthavorasit [a], Kanet Wongravee [b], Natchanun Leepipatpiboon [a,*]



1 – [6]-Gingerol

2 – Methyl [6]-gingerol

3 – [8]-Gingerol

4 – Diacetoxy-[6]-gingerdiol

5 – [10]-Gingerol

6 – Acetoxy-[8]-gingerol

7 – Diacetoxy-[8]-gingerdiol

8 – 1-Dehydro-[8]-gingerdione

9 – Methyl diacetoxy-[8]-gingerdiol

Analytical Methods (LC-UV)

TN-1139

ChromaDex

phenomenex

APPLICATIONS

HPLC-UV Analysis of Gingerol in Ginger Root

Method Status: Scientifically Valid per cGMPs for Dietary Supplements

Phenomenex: Zeshan Aqeel, J Preston, Jeff Layne
ChromaDex: Steve Baugh

Figure 2.
Analytical Reference Standards using Kinetex 5 µm Core-Shell Technology Column

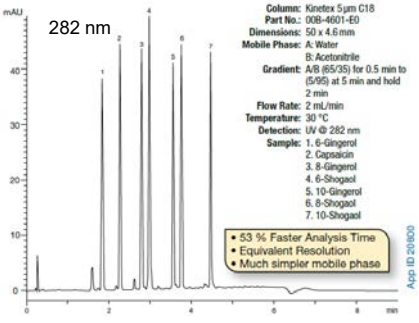
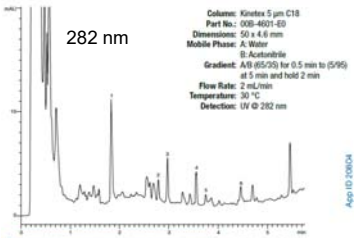


Figure 7.
Analysis of Powder Filled Gel Cap Formulations Using Kinetex® 5 µm Core-Shell Technology Column



Peaks	Compound	Conc./Capsule (mg)
1	6-Gingerol	0.144
2	8-Gingerol	0.026
3	6-Shogaol	0.05
4	10-Gingerol	0.055
5	8-Shogaol	0.012
6	10-Shogaol	0.025
	Total	0.399
	Label Claim	???

Analytical Methods (HPTLC, LC-UV)

Industrial Crops and Products 70 (2015) 238–244

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 Food Sciences, College of Life and Environmental Sciences, Konkuk University, Seoul 143-701, South Korea

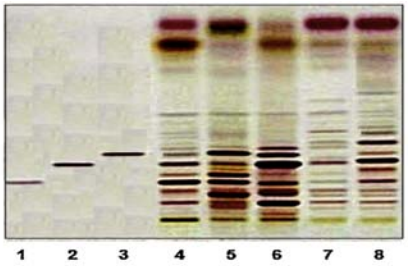


Fig. 1. HPTLC chromatograms of the tested ginger rhizome extracts, lane assignments, from left to right: standards 1: 6-shogaol, 2: 6-gingerol, 3: 6-paradol, 4: water extract, 5: ethanol extract, 6: ethyl acetate extract, 7: diethyl ether extract, 8: *n*-butanol extract.

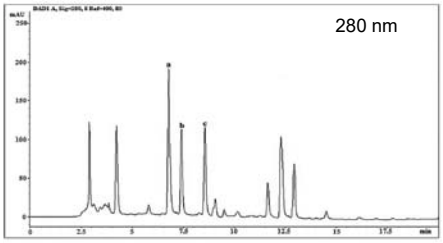


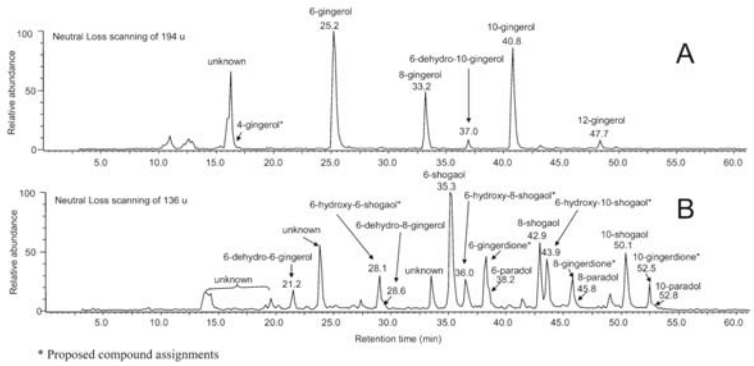
Fig. 3. HPLC analysis of ginger extract showing a: 6-gingerol, b: 6-shogaol, and c: 6-paradol.

Analytical Methods (LC-MS)

J. Agric. Food Chem. **2009**, *57*, 10014–10021
DOI:10.1021/jf9020224

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

YI TAO, WENKUI LI, WENZHONG LIANG, AND RICHARD B. VAN BREEMEN*



* Proposed compound assignments

Analytical Methods (GC-MS)

Phytochemistry 66 (2005) 1614–1635

Commercially processed dry ginger (*Zingiber officinale*): Composition and effects on LPS-stimulated PGE_2 production

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

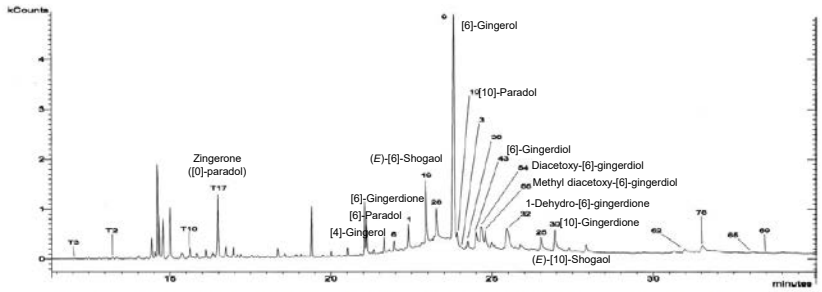
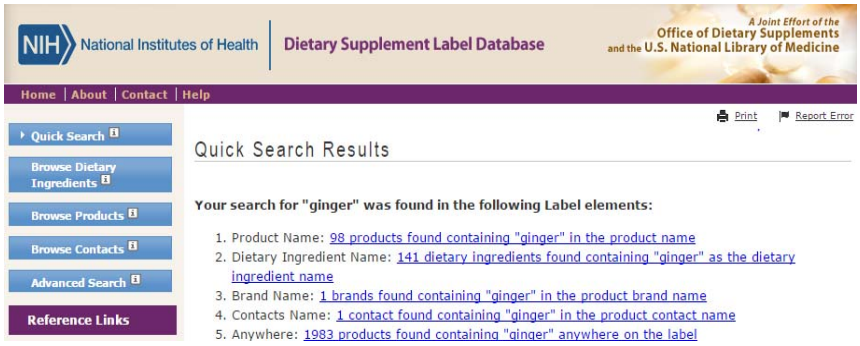


Fig. 1. GC chromatogram of original crude dichloromethane extract (X) of dry commercial ginger. Numbers refer to Tables 2 and 3.

Ginger Dietary Supplements in ODS DSLD



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

Parameter	Requirement
Analytical Range (%)	0.05 – 50
Limit of Quantitation (LOQ) (%)	≤ 0.05
Recovery (%)	90 – 107
% RSD_r	≤ 5
% RSD_R	≤ 8

SMPR: 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 D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis; http://www.eoma.aoac.org/app_d.pdf
- Appendix F: Guidelines for Standard Method Performance Requirements; http://www.eoma.aoac.org/app_f.pdf
- 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?


PLANTATION
ESSENCE JAMAICA
GINGER
ALCOHOL 93%
For Dyspepsia, Cholera Morbus, Flatulent Colic, Nausea and General Debility.

DRAFT AOAC SMPR 2016.XXX; Version 5; November 16, 2016

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]-gingerdiols, [6]-, [8]- 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.[1] 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** (SD_r) or **% repeatability relative standard deviation** ($\%RSD_r$).

[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** (SD_R) or **% reproducibility relative standard deviation** (% RSD_R).

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

Table 1: Commercial Sources of Ginger Constituents.

	Commercially Available Ginger Constituents									
	Gingerols			Shogaols			Paradols			Zingerone
	[6]-	[8]-	[10]-	[6]-	[8]-	[10]-	[6]-	[8]-	[10]-	
Chengdu Biopurify	X	X	X	X	X	X				X
Chromadex	X	X	X	X	X	X				
Extrasynthese	X	X		X						
Phytolab	X	X	X	X	X	X				
Sigma-Aldrich	X	X	X	X		X				X
Tokiwa	X	X	X	X						
Dalton Research	X	X	X	X			X	X	X	

Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements*, 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf.

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.

Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_d.pdf.

Appendix F: Guidelines for Standard Method Performance Requirements; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf.

Appendix K: Guidelines for Dietary Supplements and Botanicals; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_k.pdf.

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

Table 2: Method Performance Requirements.

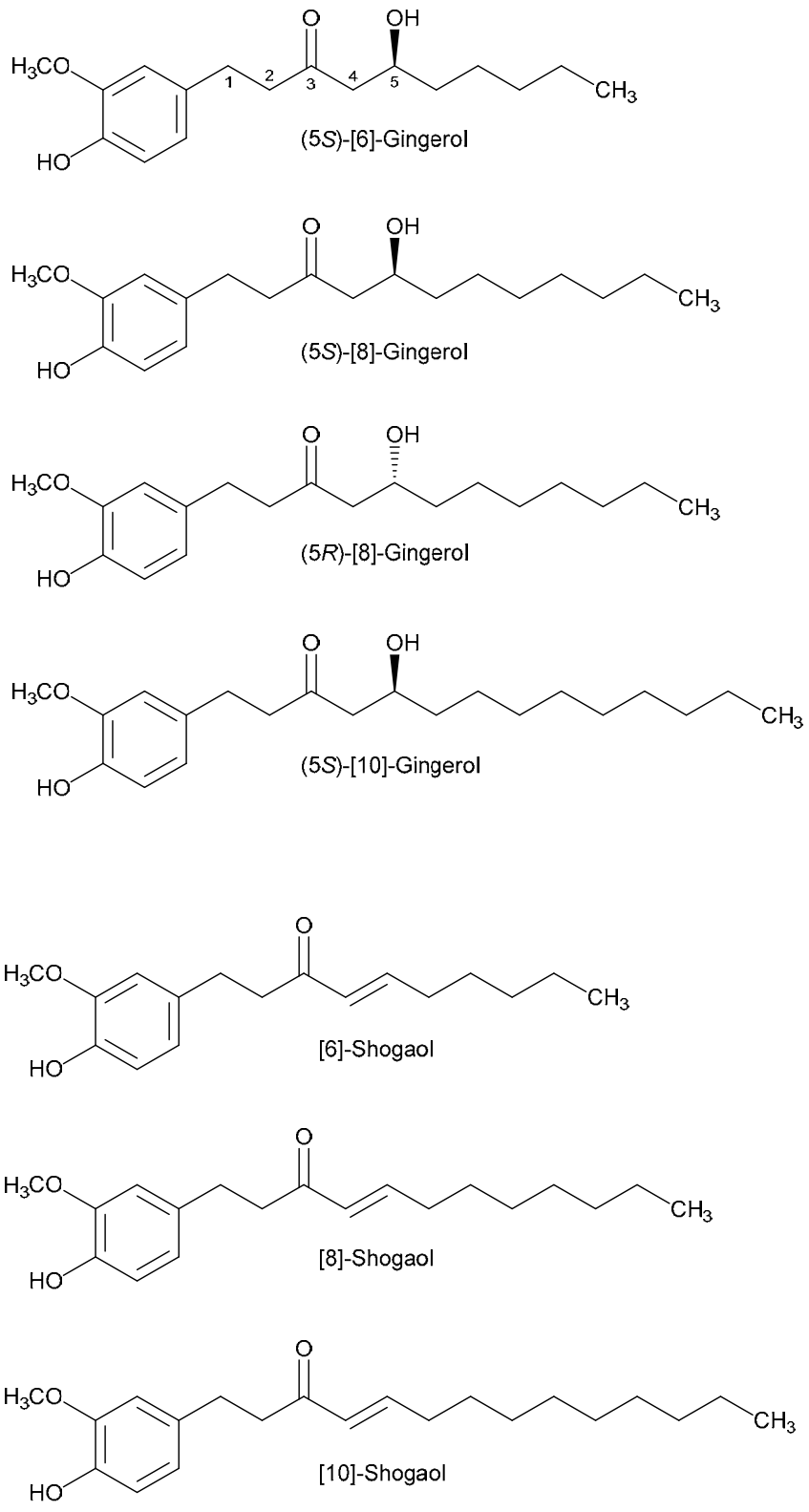
Parameter	**Requirement**
Analytical Range (%)	0.05 – 50
Limit of Quantitation (LOQ) (%)	0.05
Recovery (%)	90 – 107
% RSD_r	≤ 5
% RSD_R	≤ 8

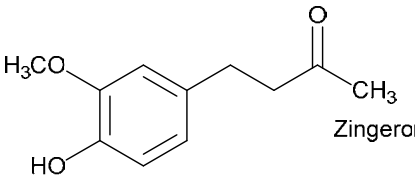
Table 3: Matrices

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

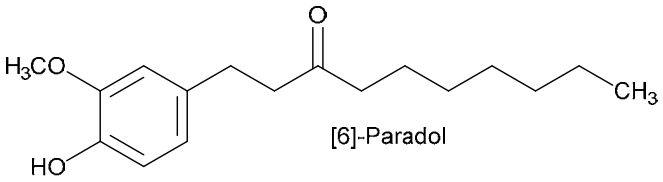
Optional:
Rhizome soft extract
Tincture
Softgel capsules

Figure 1: Chemical Structures of Gingerols, Shogaols, Paradols, Zingerone, Gingerdiones and Gingerdiols.

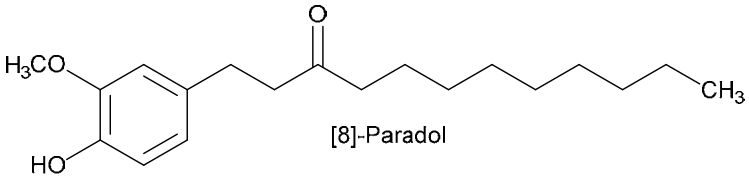




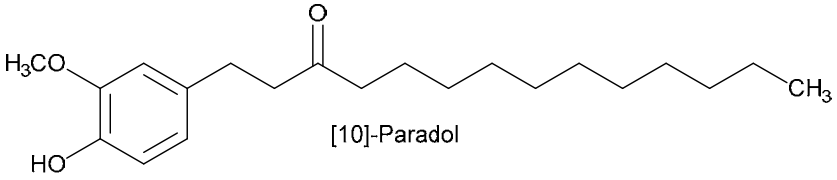
Zingerone



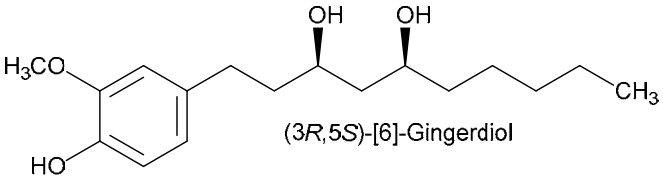
[6]-Paradol



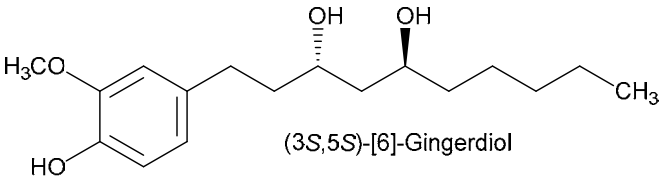
[8]-Paradol



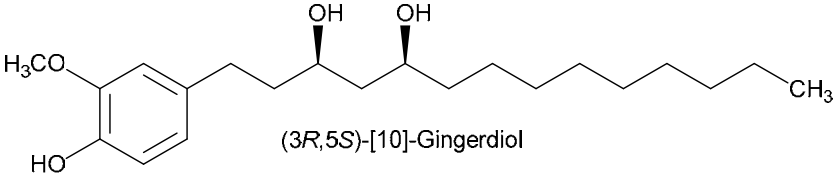
[10]-Paradol



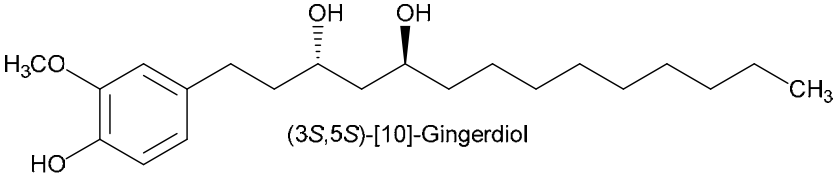
(3*R*,5*S*)-[6]-Gingerdiol



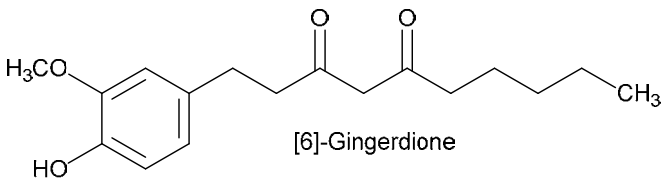
(3*S*,5*S*)-[6]-Gingerdiol



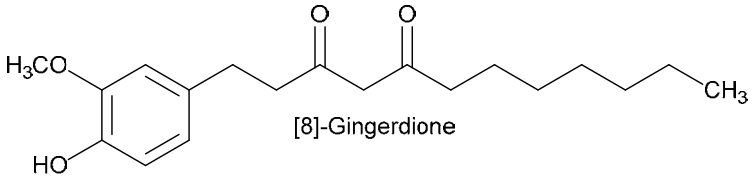
(3*R*,5*S*)-[10]-Gingerdiol



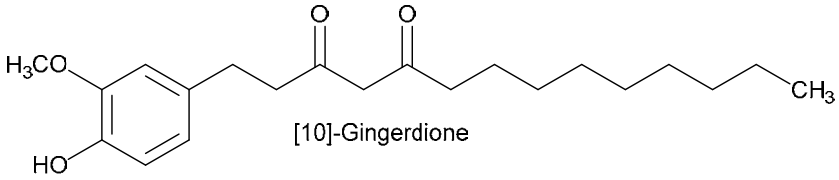
(3*S*,5*S*)-[10]-Gingerdiol



[6]-Gingerdione



[8]-Gingerdione



[10]-Gingerdione

Table 4: Analytes with Chemical Attributes and Identifiers.

Compound	IUPAC Name	Formula	CAS Number	UNII Code	InChi Key	PubChem
(*5S*)-[6]-Gingerol	**(S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one**	C17H26O4	**23513-14-6**	925QK2Z900	NLDDIKRKFXEWBK-AWEZNQCLSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/442793
(*5R*)-[6]-Gingerol	(R)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one	C17H26O4	72749-01-0		NLDDIKRKFXEWBK-CQSZACIVSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/12310197
(*5S*)-[8]-Gingerol	**(S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one**	C19H30O4	**23513-08-8**	LB0IJB138K	BCIWKKMTBRYQJU-INIZCTEOSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/168114
(*5R*)-[8]-Gingerol	(R)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one	C19H30O4	135272-33-2		BCIWKKMTBRYQJU-MRXNPFEDSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/11023711
(*5S*)-[10]-Gingerol	**(S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)tetradecan-3-one**	C21H34O4	**23513-15-7**	ND6ZLI4J0V	AIULWNKTYPZYAN-SFHVURJKSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/168115
[6]-Shogaol	(E)-1-(4-hydroxy-3-methoxyphenyl)dec-4-en-3-one	C17H24O3	555-66-8	83DNB5FIRF	OQWKEEOHDMUXEO-BQYQJAHWSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/5281794
[8]-Shogaol	(E)-1-(4-hydroxy-3-methoxyphenyl)dodec-4-en-3-one	C19H28O3	36700-45-5	AV4IK2HCNT	LGZSMXJRMTYABD-MDZDMXLPSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/6442560
[10]-Shogaol	(E)-1-(4-hydroxy-3-methoxyphenyl)tetradec-4-en-3-one	C21H32O3	36752-54-2	UP39BHE708	FADFGCOCHHNRHF-VAWYXSNFSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/6442612
Zingerone ([0]-Paradol)	4-(4-hydroxy-3-methoxyphenyl)butan-2-one	C11H14O3	122-48-5	4MMW850892	OJYLAHXKWMRDGS-UHFFFAOYSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/31211
[6]-Paradol	1-(4-hydroxy-3-methoxyphenyl)decan-3-one	C17H26O3	27113-22-0	BO24ID7E9U	CZNLTCTYLMYLHL-UHFFFAOYSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/94378
[8]-Paradol	1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one	C19H30O3	27113-23-1		TYQRTQZWHUXDLG-UHFFFAOYSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/213821
[10]-Paradol	1-(4-hydroxy-3-methoxyphenyl)tetradecan-3-one	C21H34O3	36700-48-8		XNBUKRQGYHYOOP-UHFFFAOYSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/51352076
[6]-Gingerdione	1-(4-hydroxy-3-methoxyphenyl)decane-3,5-dione	C17H24O4	61871-71-4	L2L6JCL6YY	KMNVXQHNIWUUSE-UHFFFAOYSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/162952
[8]-Gingerdione	1-(4-hydroxy-3-methoxyphenyl)dodecane-3,5-dione	C19H28O4	77334-06-6	70E1Y63Q2L	QDSRAFNZQKMHPZ-UHFFFAOYSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/14440537
[10]-Gingerdione	1-(4-hydroxy-3-methoxyphenyl)tetradecane-3,5-dione	C21H32O4	79067-90-6		QPSYZJDGMPQMSV-UHFFFAOYSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/14440539
(*3R,5S*)-[6]-Gingerdiol	**(+)-(3R,5S)-1-(4-hydroxy-3-methoxyphenyl)decane-3,5-diol**	C17H28O4	**154905-69-8**	4C9F8U79BX	QYXKQNMJTHPKBP-LSDHHAIUSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/11369949
(*3S,5R*)-[6]-Gingerdiol	(-)-(3S,5R)-1-(4-hydroxy-3-methoxyphenyl)decane-3,5-diol	C17H28O4	53318-09-5			
(*3S,5S*)-[6]-Gingerdiol	(3S,5S)-1-(4-hydroxy-3-methoxyphenyl)decane-3,5-diol	C17H28O4	143615-76-3		QYXKQNMJTHPKBP-GJZGRUSLSA-N	https://pubchem.ncbi.nlm.nih.gov/compound/15839040
(*3R,5S*)-[8]-Gingerdiol	**(3R,5S)-1-(4-hydroxy-3-methoxyphenyl)dodecane-3,5-diol**	C19H32O4	**53254-76-5**		RLBBNYBPCMIQMG-DLBZAZTESA-N	https://pubchem.ncbi.nlm.nih.gov/compound/101941698
(*3R,5S*)-[10]-Gingerdiol	**(3R,5S)-1-(4-hydroxy-3-methoxyphenyl)tetradecane-3,5-diol**	C21H36O4	**53254-77-6**		LGSIUDXMEDKEPY-RBUKOAKNSA-N	
(*3S,5R*)-[10]-Gingerdiol	(3S,5R)-1-(4-hydroxy-3-methoxyphenyl)tetradecane-3,5-diol	C21H36O4	1339934-29-0		LGSIUDXMEDKEPY-QINVSXPYNA-N	
(*3S,5S*)-[10]-Gingerdiol	(3S,5S)-1-(4-hydroxy-3-methoxyphenyl)tetradecane-3,5-diol	C21H36O4	1438241-35-0		LGSIUDXMEDKEPY-OALUTQOASA-N	https://pubchem.ncbi.nlm.nih.gov/compound/101572265

Note: Naturally prevalent stereoisomers are shown in bold: (5*S*) configuration for gingerols, (3*R*,5*S*) configuration for gingerdiols.

AOAC INTERNATIONAL STAKEHOLDER PANEL ON DIETARY SUPPLEMENTS

Garrett Zielinski, Covance Laboratories

Free Amino Acids Working Group - SMPR Presentation

March 17, 2017

Marriott Washingtonian Center, Gaithersburg, Maryland, USA

Fitness for Purpose
As Agreed September 16, 2016

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

β-alanine	Alanine	Arginine	Asparagine
Aspartic Acid	Cysteine	Cystine	Glutamic Acid
Glutamine	Glycine	Histidine	Hydroxyproline
Isoleucine	Leucine	Lysine	Methionine
Phenylalanine	Proline	Serine	Taurine
Threonine	Tryptophan	Tyrosine	Valine

SMPR Key Points

Method Performance Requirements

Parameters	Acceptable Criteria
Analytical Range (%)	0.04 - 100
LOQ (%)	≤0.04
Recommended LOD (%)	≤0.01
For individual free amino acid components measured.	

Ranges (%)	0.04 -10	> 10
Recovery (%)	90 - 107	98 – 102
% RSD_r	≤ 5	≤ 3
% RSD_R	≤ 8	≤4
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?

DRAFT AOAC Free Alpha Amino Acids SMPR, v7, 7 March 2017.

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.

[1]Federal 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 reproducibilitystandard deviation (SD_R); or % reproducibilityrelative 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):**

Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements*, 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

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 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.

Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_d.pdf

Appendix F: Guidelines for Standard Method Performance Requirements; 19thEdition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

Appendix K**:** Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis (2016) 20th Ed., AOAC INTERNATIONAL.

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

102 **Table 1: Free alpha amino acids and related compounds**

Common name	IUPAC Systematic Name	CAS No.*
β-alanine	3-aminopropanoic acid	107-95-9
alanine	2-aminopropanoic acid	302-72-7
arginine	2-amino-5-(diaminomethylideneamino)pentanoic acid	2500-25-7
asparagine	2,4-diamino-4-oxobutanoic acid	3130-87-8
aspartic acid	2-aminobutanedioic acid	617-45-8
cysteine	2-amino-3-sulfanylpropanoic acid	3374-22-9
cystine	2-amino-3-[[(2R)-2-amino-2-carboxyethyl]disulfanyl]propanoic acid	923-32-0
glutamic acid	2-aminopentanedioic acid	617-65-2
glutamine	2,5-diamino-5-oxopentanoic acid	585-21-7
glycine	2-aminoethanoic acid	56-40-6
Histidine	2-amino-3-(1H-imidazol-5-yl)propanoic acid	4998-57-6
Hydroxyproline	4-hydroxypyrrolidine-2-carboxylic acid	51-35-4
isoleucine	2-amino-3-methylpentanoic acid	443-79-8
leucine	2-amino-4-methylpentanoic acid	328-39-2
lysine	2,6-diaminohexanoic acid	70-54-2
methionine	2-amino-4-methylsulfanylbutanoic acid	59-51-8
phenylalanine	2-amino-3-phenylpropanoic acid	63-91-2
proline	pyrrolidine-2-carboxylic acid	609-36-9
serine	2-amino-3-hydroxypropanoic acid	302-84-1
taurine	2-aminoethanesulfonic acid	107-35-7
threonine	2-amino-3-hydroxybutanoic acid	80-68-2
tryptophan	2-amino-3-(1H-indol-3-yl)propanoic acid	54-12-6
tyrosine	2-amino-3-(4-hydroxyphenyl)propanoic acid	556-03-6
valine	2-amino-3-methylbutanoic acid	516-06-3
*CAS numbers specify the racemic forms, except for glycine and taurine which are achiral.		

103
104

Table 2 : Dietary Ingredients and Supplements

Powder
Tablets
Liquids
Capsules

Table 3: Method performance requirements (part 1)

Parameters	**Acceptable Criteria**
Analytical Range (%)	0.04 - 100
LOQ (%)	≤0.04
Recommended LOD (%)	≤0.01
For individual free amino acid components measured.	

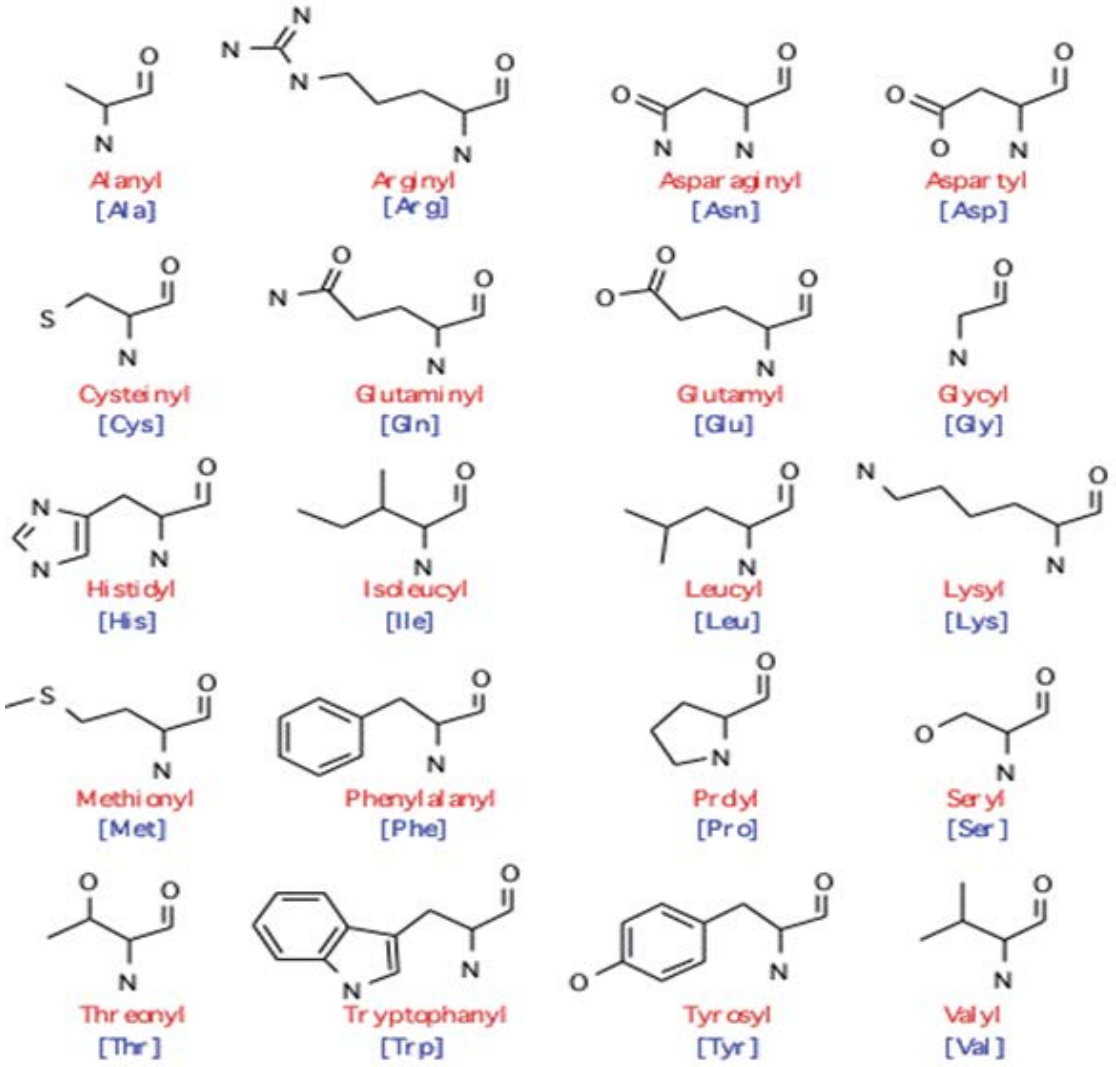
Table 4: Method performance requirements (part 2)

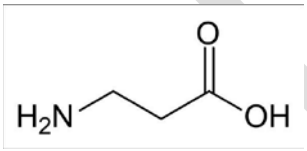
Ranges (%)	0.04 -10	> 10
Recovery (%)	90 - 107	98 – 102
% RSD_r	≤ 5	≤ 3
% RSD_R	≤ 8	≤4
For individual free amino acid components measured		

Table 5 : Non-target Compounds

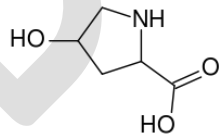
Norvaline
Sarcosine
Carnitine
Citrulline
Ornithine
Selenomethionine
GABA
Selenocystine
5HTP

Figure 1 : Molecular structures of free amino acids and related compounds identified in table 1.





β-alanine



hydoxyproline

AOAC INTERNATIONAL STAKEHOLDER PANEL ON DIETARY SUPPLEMENTS

Inger Reidun Aukrust, Kappa Bioscience
Vitamins K_1 and K_2 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 (phylloquinone) 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-coateds 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:

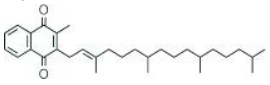
- **Vitamin K1** (phylloquinone, defined as the sum of *cis* and *trans* isomers)
- **Vitamin K2** (the menaquinone series, MK4 through MK14).

MK4 and MK7 are the most well-studied menaquinones.

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

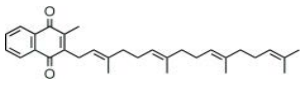
Background on Vitamin K

Vitamin K1 (phylloquinone)



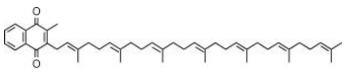
- Made by plants and algae-
- Only 5-10% of ingested K1 reaches circulation

Vitamin K2 - Menaquinone 4



- Pharmacokinetics like K1
- Used in many studies due to commercial availability

Vitamin K2 - Menaquinone 7



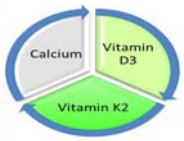
- Found in certain fermented foods
- Readily absorbed (nearly 100%) and distributed to several tissues

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 *cis* and *trans* forms of vitamin K1, all-trans forms of both MK4 and MK7 (vitamin K2)
- Determine area % for total *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

- Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_d.pdf

- Appendix K: Guidelines for Dietary Supplements and Botanicals 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Also at: . AOAC Int. 95, 268(2012); DOI: 10.5740/jaoacint.11-447 and available at: http://www.eoma.aoac.org/app_k.pdf

Analytical Range & LOQ Requirements based on Matrix

	Vitamin K_1 & K_2*	
Parameter	Dietary Supplements	Dietary Ingredients
Analytical range	1– 3000 ppm	1,000 – 1M ppm
Limit of Quantitation	0.5 ppm	200 ppm

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

Recovery, Repeatability & Reproducibility

Parameter	Range*		
	1 – 100 ppm	>100 – 3,000	>3,000 ppm
Recovery (%)	80 – 110	90-107	97 – 103
% RSD_r	< 11	< 6	< 5
% RSD_R	< 15	< 8	< 6

* 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_1 and K_2 in Dietary Supplements and* Dietary *Ingredients* as presented.

Discussion?

DRAFT AOAC SMPR 2016.XXX; Version 5; December 5, 2016

Method Name: **Determination of Vitamins K_1 and K_2 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 K_1 (phylloquinone); all -*trans* forms of both MK-4 and MK-7 (vitamin K_2); and determine area % for total *cis* forms of Vitamin K_2 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 deviation (%RSD_r).

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

Vitamin K_1 .— Phyilloquinone. IUPAC name: 2-methyl-3-[(2*E*)-3,7,11,15-tetramethyl hexadec-2-en-1-yl]naphthoquinone. CAS number: 084-80-0. See figure 1.

Vitamin K_2 .— 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

	Vitamin K_1 & K_2*	
Parameter	Dietary Supplements	Dietary Ingredients
Analytical range	1– 3000 ppm	1,000 – 1M ppm
Limit of Quantitation	0.5 ppm	200 ppm

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

Table 2: Method Performance Requirements as a Function of Range

Parameter	**Range***		
	1 – 100 ppm	>100 – 3,000	>3,000 ppm
Recovery (%)	80 – 110	90-107	97 – 103
% RSD_r	< 11	< 6	< 5
% RSD_R	< 15	< 8	< 6

* 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

Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements*, 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_f.pdf

7. **Validation Guidance**:

All target analytes (vitamin K_1, 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.

Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available at: http://www.eoma.aoac.org/app_d.pdf

Appendix K: Guidelines for Dietary Supplements and Botanicals 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Also at: . AOAC Int. 95, 268(2012); DOI: 10.5740/jaoacint.11-447 and available at: http://www.eoma.aoac.org/app_k.pdf

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

Figure 1: Molecular structures of vitamin K_1 and K_2

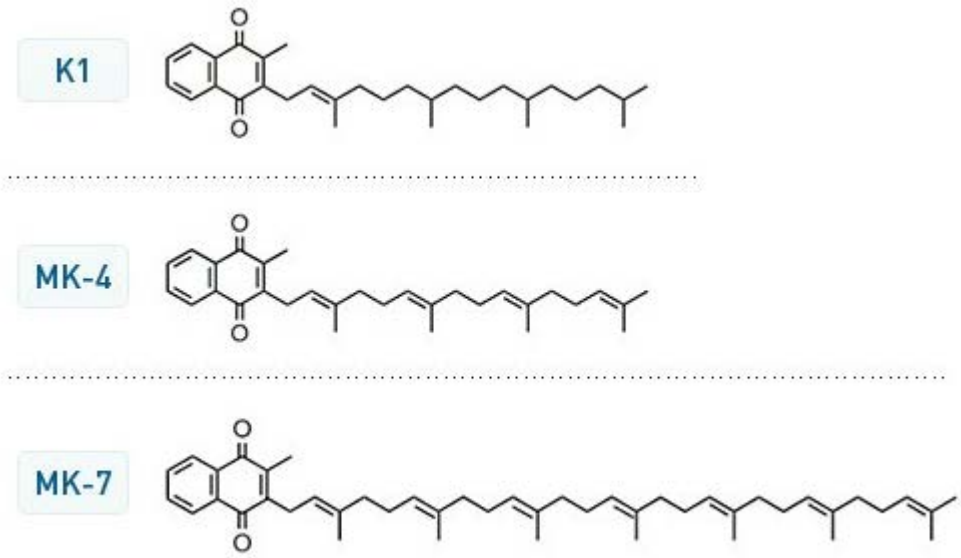


Table 3: Matrices

Dietary Ingredients:

powders
oils
extracts
encapsulated

Dietary Supplements :

powders
tablets
gummies
oils
liquids
capsules
softgel capsules
tinctures
gelcaps
chewables

Draft, Do Not Distribute

AOAC Stakeholder Panel on Dietary Supplements
2016 Advisory Panel Meeting

Meeting Minutes
Thursday, December 15, 2016, 8:30 a.m. – 12:00 p.m. ET

Attendees

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

Darryl Sullivan, Covance; Chair
Gisele Atkinson, CRN
Joseph Betz, NIH - ODS
Peter Chang, Herbalife
Gabriel Giancaspro, USP
Adam Kuszak, NIH – ODS
Maged Sharaf, AHPA
Sibyl Swift, FDA
John Travis, NSF International

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

Scott Coates
Christopher Dent
Dawn Frazier
Deborah McKenzie
Tien Milor
Robert Rathbone

Meeting Minutes

I. Welcome and Introductions

All were introduced and roll taken at 8:35 am ET.

II. Ingredient Updates

Frazier reviewed the status of Standard Method Performance Requirements® (SMPRs®) and methods for each ingredient that has been addressed by SPDS so far:

- Anthocyanins: 1 SMPR, 0 First Action *Official Methods of Analysis*℠
- Chondroitin: 2 SMPRs, 1 First Action *Official Method of Analysis*
- PDE5 Inhibitors: 3 SMPRs, 1 First Action *Official Method of Analysis*
- Ashwagandha: 1 SMPR, 1 First Action *Official Method of Analysis*
- Cinnamon: 1 SMPR, 0 First Action *Official Methods of Analysis*
- Folin C: 1 SMPR, 0 First Action *Official Methods of Analysis.* Teleconference held with Working Group Chair John Finley, LSU at which time it was agreed that the Call for Methods required wider distribution, which he offered to assist AOAC with. Folin C Call for Methods will be issued before the end of December, 2016.
- Kratom: 1 SMPR, 0 First Action *Official Methods of Analysis*
- Aloin: 1 SMPR, 1 First Action *Official Method of Analysis*
- Tea: 1 SMPR, 1 First Action *Official Method of Analysis*
- Vitamin D: 1 SMPR, no methods submitted. SMPR revision has been authorized by SPDS and completed by the Vitamin D Working Group. Vote on revised SMPR scheduled for March, 2017 SPDS Meeting. Call for methods will follow.

- Collagen: 1 SMPR. No methods submitted. Teleconference held with Working Group Chair Jason Cooley, BioCell, at which time it was determined that this SMPR may be asking too much of one method. Cooley recommended revisions to this SMPR.
- Lutein: 1 SMPR. 2 methods submitted, to be reviewed by an AOAC Expert Review Panel on the afternoon of 12/15/2016.
- Turmeric: 1 SMPR. 2 methods submitted, to be reviewed by an AOAC Expert Review Panel on the afternoon of 12/15/2016.
- Protein: 4 SMPRs, Call for Methods to be issued.
- Vitamin B12: 1 SMPR, Call for Methods to be issued.
- SMPRs for Aloe Vera, Free Amino Acids, Ginger, and Vitamins K1 and K2 are currently under development.

Betz encouraged AOAC Staff to continue to do literature searches for ingredients for which no methods are being submitted or approved. The advisory panel also agreed that they need to be clearer on exactly what types of SMPRs are being requested.

III. Next 6 Ingredients

Frazier reviewed the results of the survey that was provided to advisory panel members in a presentation.[1] The presentation concluded with a summary slide as follows:

Ingredient	# of Recommendations
Açaí	2
Grapeseed Extract	2
Resveratrol	2
Green Tea Extract	1
Scullcap	1
Pommegranite	1
Stevia	1
SAMe	1
Jujube	1
Ochratoxin A (OTA) in licorice and astragalus	1
Hepatotoxic Pyrrolizidine alkaloids in honey and plant products.	1
ginsenosides in ginseng	1
phenolic constituents of Echinacea	1
Determination of a neurotoxic amino acid in cyanobacteria	1
Determination of flavonolignans in milk thistle	1
Determination of flavonoids in Hawthorn leaves and products	1
Determination of anthocyanins in cranberry fruit and products	1
Kavalactones	1

The panel continued discussions on the need for standards and methods for each of the various ingredients and began to prioritize for Set 7 (March, 2017 launch) and Set 8 (September, 2017 launch). Following a thorough discussion, the panel developed and agreed to the following list:

- **Echinacea (Set 7):** Methods for quantitative determination of selected phenolic marker compounds in plant materials, dietary supplements and / or dietary ingredients.

[1] Priority Ingredient Survey 2016

- **Ginsenosides in Ginseng (Set 7):** Methods for quantitative determination of selected ginsenosides in plant materials, dietary supplements and / or dietary ingredients.
- **SAMe (Set 7) :** 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.
- ***Açaí (Set 8):*** *Quantitative determination of selected anthocyanins in Açaí.*
- **Kavalactones (Set 8):** Methods for quantitative determination of selected kavalactones in plant materials, dietary supplements and / or dietary ingredients.
- **Resveratrol (Set 8):** Methods for quantitative determination of resveratrol isomers in dietary ingredients and dietary supplements.
- **Scullcap (BACKUP for Set 8):** Quantitative determination of selected marker compounds and/or negative marker compounds. (Germander)

Although the panel understood the importance of standards for açai, there were questions about its viability as an SPDS ingredient. Atkinson had submitted a paper by Alexander Schauss, AIBMR Life Sciences discussing the subject. The Advisory Panel agreed to choose açai as a Set 8 ingredient on the condition that AOAC further investigate the need for standards in this area and whether or not methods already exist. ACTION for AOAC to discuss this further with Atkinson and Schauss and report back to the Advisory Panel on this matter at the spring Advisory Panel teleconference. ACTION for Atkinson to do an email introduction for Frazier and Schauss. Scullcap was chosen as a backup ingredient if the panel decides not to move forward on açai. The group then held a brief discussion on potential working group members, chairs, and/or organizations that should be included in these new working groups.

IV. Next Steps

Frazier advised that the immediate next steps for the new ingredients will be to assign chairs for the Set 7 working groups and get them started on a launch presentation. ACTION for Frazier to begin contacting the individuals mentioned earlier in this meeting. Frazier said that the next meeting of the SPDS will be in Gaithersburg, MD and will be on March 17, 2017. At that time, SMPRs for Aloe Vera, Free Amino Acids, Ginger, and Vitamin K will be presented for approval. Further, the Set 7 Working Groups will be launched.

V. Adjourn

The group agreed to the plan of action. Actions were assigned and the meeting adjourned at approximately 12:00 pm, ET.

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



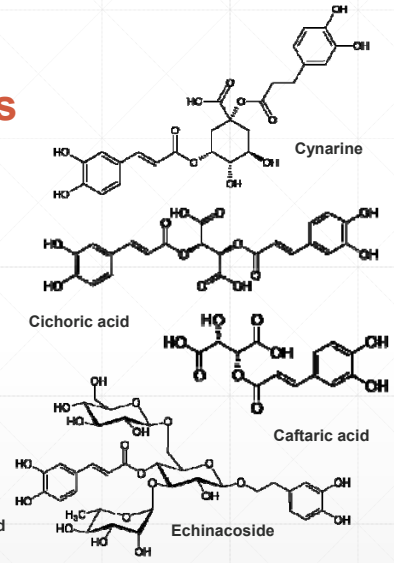
Background on the Plant Material (continued)

- 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

	Cichoric acid	Caftaric acid	Echinacoside	Chlorogenic acid	Cynarine[1]
E. angustifolia	<LOD – 0.05	<LOD – 0.02	0.13 – 1.70	<LOD – 0.15	0.07 – 0.34
E. pallida	<LOD – 0.22	0.01 – 0.08	0.13 – 1.27	<LOD – 0.30	<LOD
E. purpura root	0.33 – 2.78	0.35 – 0.80	<LOD	<LOD – 0.19	<LOD
E. purpurea tops	0.52 – 2.20	0.18 – 0.85	<LOD	<LOD – 0.03	<LOD



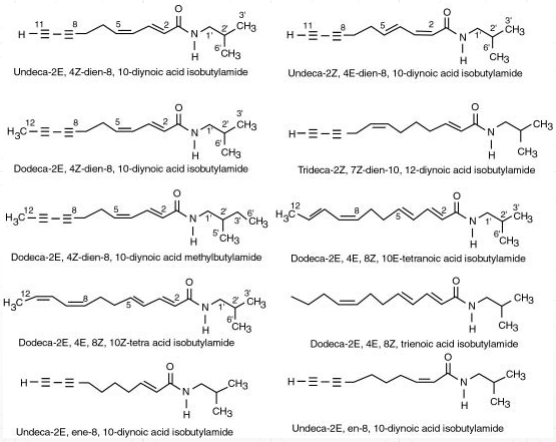
[1]Syn. 1,3-dicaffeoylquinic acid
(1*R*,3*R*,4*S*,5*R*)-1,3-bis[[(*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-4,5-dihydroxycyclohexane-1-carboxylic acid

References: Brown *et al.* 2011, *JAOAC Int*. 94(5): 1400–1410; Perry *et al.* 2001, *J Agric Food Chem*. 49(4): 1702-1706; Laasonen *et al.* 2002, *Planta Med*. 68: 572 – 574; Pellati *et al.* 2005, *Phytochem Anal*. 16(2): 77 – 85. Blaschek. In: Wichtl – Teedrogen und Phytopharmaka, 2016.

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

Total alkylamide concentrations in % of dried plant part

	Alkylamides
E. angustifolia	0.01 – 0.15
E. pallida	not present
E. purpura root	0.01 – 2.77
E. purpurea tops	0.02 – 0.53



References: Wills and Stuart 1999. *Food Chem*. 67(4): 385-388; Qu *et al*. 2005, *Hort Science*. 40(5): 1239-1242; Blaschek. In: Wichtl – Teedrogen und Phytopharmaka, 2016.

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

Reference: Karsch-Völk et al., 2015. *Cochrane Database of Systematic Reviews* 2014, Issue 2. Art. No.: CD000530

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

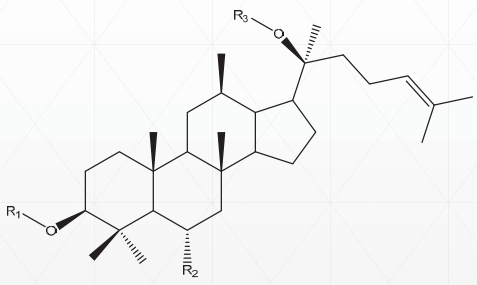
Quantitation of phenolic compounds (i.e., caftaric acid, chlorogenic acid, cichoric acid, cynarine, and echinacoside) in *Echinacea angustifolia*, *Echinacea pallida*, and *Echinacea purpurea* raw materials and finished dietary supplement products

Stakeholder Panel on Dietary Supplements: Background and Fitness for Purpose for 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:

  Brown. JAOAC Int. 2011 Sep-Oct; 94(5): 1391-9.

  Brown & Yu. JAOAC Int. 2013 Jan-Feb; 96(1): 12-19.
- Hydrolysis step to convert malonyl ginsenosides to their neutral couterparts 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 porducts

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.
  - Liu *et al.* J Pharm Biomed Anal. 2017 Feb 20;135:1761-185.
  - Xu *et al.* Nat Prod Res 2015;29(1):46-52.
  - Park *et al.* J Ginseng Res. 2013 Oct;37(4):457-67.
  - Liang *et al.* J Chromatogr A. 2013 Jul 5;1297:29-36.
- 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 *SAMe*

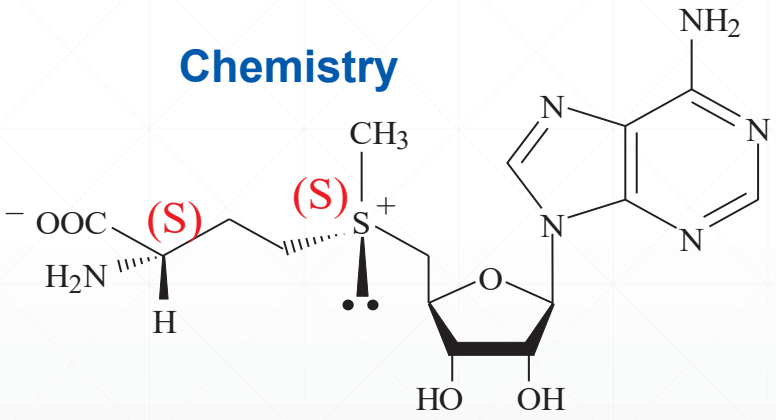
Joseph Zhou, Ph.D.
Sunshineville Health Products, Inc
AOAC Meeting Gaithersburg, Maryland
March 17, 2017

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

(1)

SAMe
Bio-Inactive, (R,S)

Reversible,
Insignificant

Degradation

(2)

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

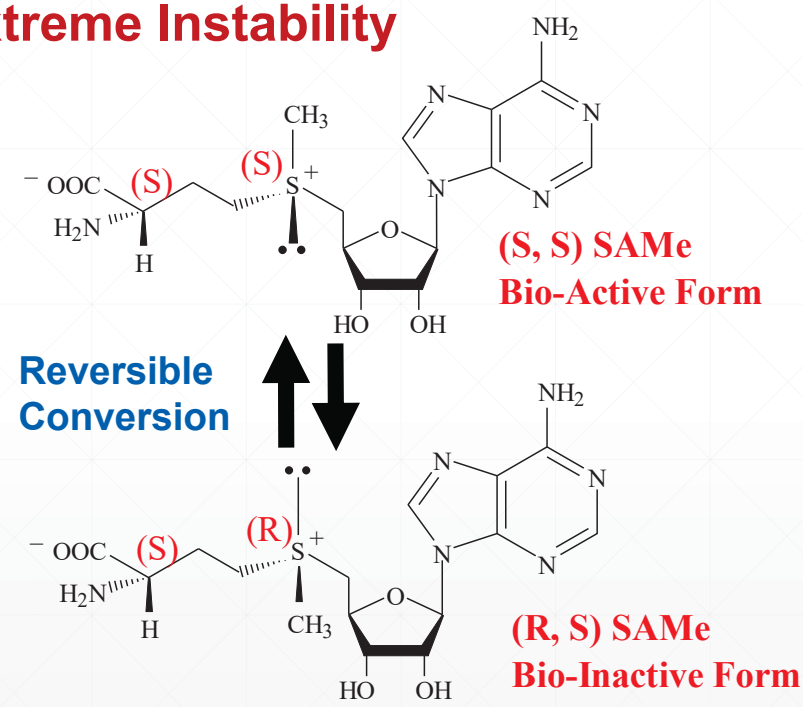
Irreversible, Significant

Challenge: SAMe's Extreme Instability

(1) Racemization

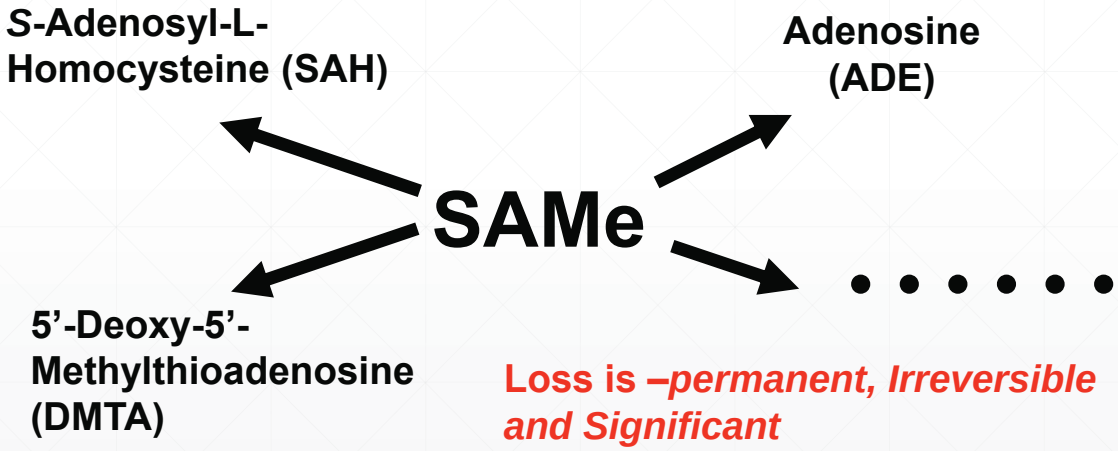
Positive Points about (R, S) SAMe:

- **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).**



Challenge: SAMe's Extreme Instability

(2) Degradation



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

Challenge: SAMe's Extreme Instability

Techniques to Reduce SAMe Product Degradation

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

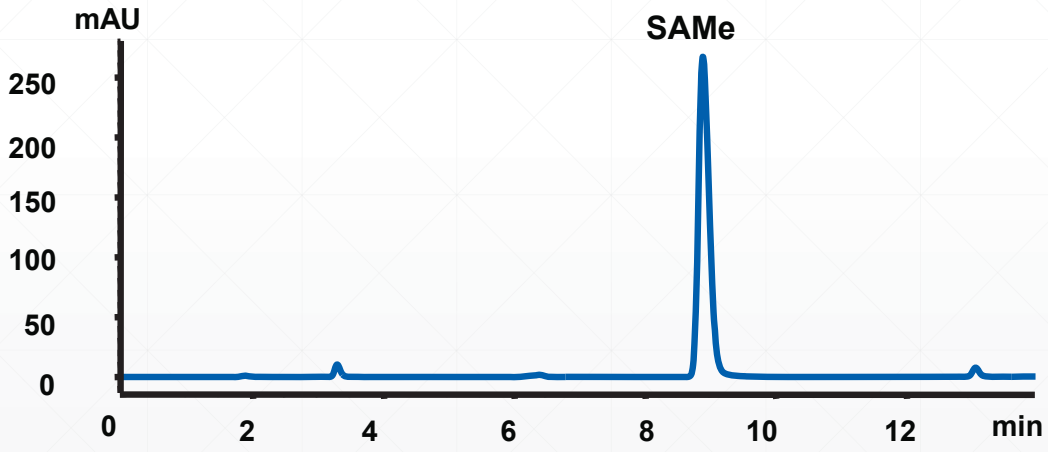
Existing Methods (General)

HPLC Method for Potency and Purity Test

HPLC :	Regular System
Column :	XTerra RP_8, 5μ, 4.6x 250mm
UV Detection:	257 nm
Mobile Phases	A: 25 mM NaH_2PO_4 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)

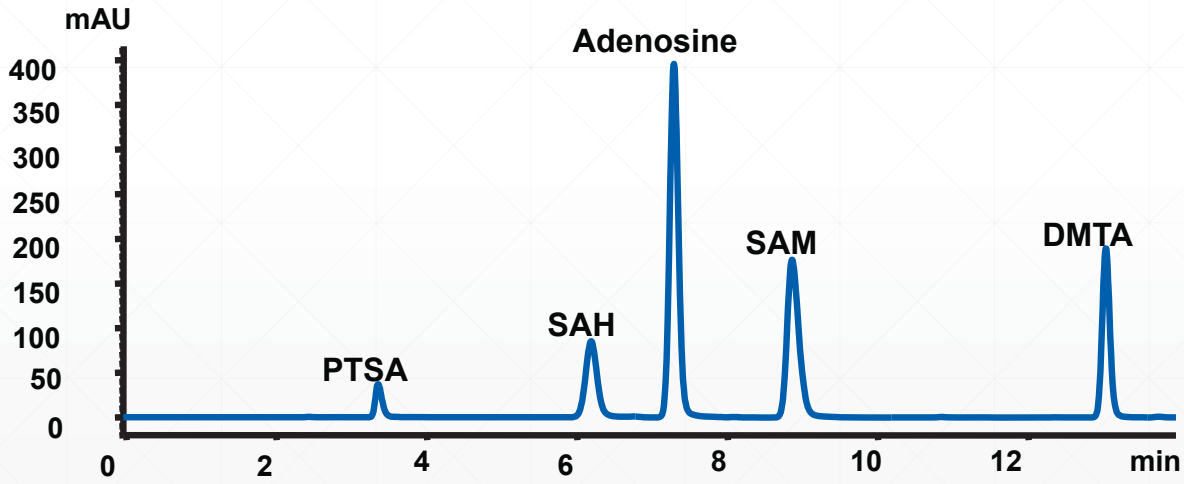
HPLC Method for Potency and Purity Test

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

Existing Methods (General)

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.

SPDS

2016 AOAC ANNUAL MEETING, SEPTEMBER 16-17

STAKEHOLDER PANEL ON DIETARY SUPPLEMENTS

RESOURCES

SPDS Key Staff Contacts:

Name	Role	Email	Telephone
Scott Coates	AOAC Chief Scientific Officer	scoates@aoac.org	301.924.7077 x 137
Christopher Dent	Standards Development Project Coordinator	cdent@aoac.org	301.924.7077 x 119
Dawn Frazier	Executive, Scientific Business Development	dfrazier@aoac.org	301.924.7077 x 117
Deborah McKenzie	Sr. Director, Standards Development and Method Approval Processes	dmckenzie@aoac.org	301.924.7077 x 157

Key Volunteer Contacts:

Name	Role	Email	Telephone
Darryl Sullivan	Chair, SPDS	darryl.sullivan@covance.com	(608) 242-2711
Brian Schaneberg	Vice Chair, SPDS	bschaneb@starbucks.com	(206) 318-0900

Useful Web Links:

AOAC Website: http://www.aoac.org

SPDS Microsite: http://goo.gl/rYwpAq

SPDS Standards Development: **Working Group Sign Up**: https://form.jotform.com/70186149225961

SPDS Conformity Assessment: Call for Experts / **ERP Application**: https://goo.gl/rWimqq

SPDS Conformity Assessment: ALL Open **Calls for Methods**: https://goo.gl/eXk9Fu

See you in Atlanta!

AOAC ANNUAL MEETING & EXPOSITION
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Appendix W

POLICY AND PROCEDURES ON VOLUNTEER CONFLICT OF INTEREST

Statement of Policy

While it is not the intention of AOAC INTERNATIONAL (AOAC) to restrict the personal, professional, or proprietary activities of AOAC members nor to preclude or restrict participation in Association affairs solely by reason of such activities, it is the sense of AOAC that conflicts of interest or even the appearance of conflicts of interest on the part of AOAC volunteers should be avoided. Where this is not possible or practical under the circumstances, there shall be written disclosure by the volunteers of actual or potential conflicts of interest in order to ensure the credibility and integrity of AOAC. Such written disclosure shall be made to any individual or group within the Association which is reviewing a recommendation which the volunteer had a part in formulating and in which the volunteer has a material interest causing an actual or potential conflict of interest.

AOAC requires disclosure of actual or potential conflicts of interest as a condition of active participation in the business of the Association. The burden of disclosure of conflicts of interest or the appearance of conflicts of interest falls upon the volunteer.

A disclosed conflict of interest will not in itself bar an AOAC member from participation in Association activities, but a three-fourths majority of the AOAC group reviewing the issue presenting the conflict must concur by secret ballot that the volunteer's continued participation is necessary and will not unreasonably jeopardize the integrity of the decision-making process.

Employees of AOAC are governed by the provision of the AOAC policy on conflict of interest by staff. If that policy is in disagreement with or mute on matters covered by this policy, the provisions of this policy shall prevail and apply to staff as well.

Illustrations of Conflicts of Interest

1. A volunteer who is serving as a committee member or referee engaged in the evaluation of a method or device; who is also an employee of or receiving a fee from the firm which is manufacturing or distributing the method or device or is an employee of or receiving a fee from a competing firm.

2. A volunteer who is requested to evaluate a proposed method or a related collaborative study in which data are presented that appear detrimental (or favorable) to a product distributed or a position supported by the volunteer's employer.

3. A referee who is conducting a study and evaluating the results of an instrument, a kit, or a piece of equipment which will be provided gratis by the manufacturer or distributor to one or more of the participating laboratories, including his or her own laboratory, at the conclusion of the study.

4. Sponsorship of a collaborative study by an interest (which may include the referee) which stands to profit from the results; such sponsorship usually involving the privilege granted by the investigator to permit the sponsor to review and comment upon the results prior to AOAC evaluation.

5. A volunteer asked to review a manuscript submitted for publication when the manuscript contains information which is critical of a proprietary or other interest of the reviewer.

The foregoing are intended as illustrative and should not be interpreted to be all-inclusive examples of conflicts of interest AOAC volunteers may find themselves involved in.

Do's and Don't's

Do avoid the appearance as well as the fact of a conflict of interest.

Do make written disclosure of any material interest which may constitute a conflict of interest or the appearance of a conflict of interest.

Do not accept payment or gifts for services rendered as a volunteer of the Association without disclosing such payment or gifts.

Do not vote on any issue before an AOAC decision-making body where you have the appearance of or an actual conflict of interest regarding the recommendation or decision before that body.

Do not participate in an AOAC decision-making body without written disclosure of actual or potential conflicts of interest in the issues before that body.

Do not accept a position of responsibility as an AOAC volunteer, without disclosure, where the discharge of the accepted responsibility will be or may appear to be influenced by proprietary or other conflicting interests.

Procedures

Each volunteer elected or appointed to an AOAC position of responsibility shall be sent, at the time of election or appointment, a copy of this policy and shall be advised of the requirement to adhere to the provisions herein as a condition for active participation in the business of the Association. Each volunteer, at the time of his or her election or appointment, shall indicate, in writing, on a form provided for this purpose by AOAC, that he or she has read and accepts this policy.

Each year, at the spring meeting of the AOAC Board of Directors, the Executive Director shall submit a report certifying the requirements of this policy have been met; including the names and positions of any elected or appointed volunteers who have not at that time indicated in writing that they have accepted the policy.

Anyone with knowledge of specific instances in which the provisions of this policy have not been complied with shall report these instances to the Board of Directors, via the Office of the Executive Director, as soon as discovered.

* * * * * *

Adopted: March 2, 1989
Revised: March 28, 1990
Revised: October 1996
Reviewed by outside counsel March 2000 (Fran Dwornik) and found to be current and relevant

Appendix U

ANTITRUST POLICY STATEMENT AND GUIDELINES

Introduction

It is the policy of AOAC INTERNATIONAL (AOAC) and its members to comply strictly with all laws applicable to AOAC activities. Because AOAC activities frequently involve cooperative undertakings and meetings where competitors may be present, it is important to emphasize the on-going commitment of our members and the Association to full compliance with national and other antitrust laws. This statement is a reminder of that commitment and should be used as a general guide for AOAC and related individual activities and meetings.

Responsibility for Antitrust Compliance

The Association's structure is fashioned and its programs are carried out in conformance with antitrust standards. However, an equal responsibility for antitrust compliance -- which includes avoidance of even an appearance of improper activity -- belongs to the individual. Even the appearance of improper activity must be avoided because the courts have taken the position that actual proof of misconduct is not required under the law. All that is required is whether misconduct can be inferred from the individual's activities.

Employers and AOAC depend on individual good judgment to avoid all discussions and activities which may involve improper subject matter and improper procedures. AOAC staff members work conscientiously to avoid subject matter or discussion which may have unintended implications, and counsel for the Association can provide guidance with regard to these matters. It is important for the individual to realize, however, that the competitive significance of a particular conduct or communication probably is evident only to the individual who is directly involved in such matters.

Antitrust Guidelines

In general, the U.S. antitrust laws seek to preserve a free, competitive economy and trade in the United States and in commerce with foreign countries. Laws in other countries have similar objectives. Competitors (including individuals) may not restrain competition among themselves with reference to the price, quality, or distribution of their products, and they may not act in concert to restrict the competitive capabilities or opportunities of competitors, suppliers, or customers.

Although the Justice Department and Federal Trade Commission generally enforce the U.S. antitrust laws, private parties can bring their own lawsuits. Penalties for violating the U.S. and other antitrust laws are severe: corporations are subject to heavy fines and injunctive decrees, and may have to pay substantial damage judgments to injured competitors, suppliers, or customers. Individuals are subject to criminal prosecution, and will be punished by fines and imprisonment. Under current U.S. federal sentencing guidelines, individuals found guilty of bid rigging, price fixing, or market allocation must be sent to jail for at least 4 to 10 months and must pay substantial minimum fines.

Since the individual has an important responsibility in ensuring antitrust compliance in AOAC activities, everyone should read and heed the following guidelines.

1. Don't make any effort to bring about or prevent the standardization of any method or product for the purpose or intent of preventing the manufacture or sale of any method or product not conforming to a specified standard
2. Don't discuss with competitors your own or the competitors' prices, or anything that might

affect prices such as costs, discounts, terms of sale, distribution, volume of production, profit margins, territories, or customers.

3. Don't make announcements or statements at AOAC functions, outside leased exhibit space, about your own prices or those of competitors.

4. Don't disclose to others at meetings or otherwise any competitively sensitive information.

5. Don't attempt to use the Association to restrict the economic activities of any firm or any individual.

6. Don't stay at a meeting where any such price or anti-competitive talk occurs.

7. Do conduct all AOAC business meetings in accordance with AOAC rules. These rules require that an AOAC staff member be present or available, the meeting be conducted by a knowledgeable chair, the agenda be followed, and minutes be kept.

8. Do confer with counsel before raising any topic or making any statement with competitive ramifications.

9. Do send copies of meeting minutes and all AOAC-related correspondence to the staff member involved in the activity.

10. Do alert the AOAC staff to any inaccuracies in proposed or existing methods and statements issued, or to be issued, by AOAC and to any conduct not in conformance with these guidelines.

Conclusion

Compliance with these guidelines involves not only avoidance of antitrust violations, but avoidance of any behavior which might be so construed. Bear in mind, however, that the above antitrust laws are stated in general terms, and that this statement is not a summary of applicable laws. It is intended only to highlight and emphasize the principal antitrust standards which are relevant to AOAC programs. You must, therefore, seek the guidance of either AOAC counsel or your own counsel if antitrust questions arise.

Adopted by the AOAC Board of Directors: September 24, 1989
Revised: March 11, 1991
Revised October 1996

Appendix V

POLICY ON THE USE OF THE ASSOCIATION NAME, INITIALS, IDENTIFYING INSIGNIA, LETTERHEAD, AND BUSINESS CARDS

Introduction

The following policy and guidelines for the use of the name, initials, and other identifying insignia of AOAC INTERNATIONAL have been developed in order to protect the reputation, image, legal integrity and property of the Association.

The name of the Association, as stated in its bylaws, is "AOAC INTERNATIONAL". The Association is also known by its initials, AOAC, and by its logo, illustrated below, which incorporates the Association name and a representation of a microscope, book, and flask. The AOAC logo is owned by the Association and is registered with the U.S. Patent and Trademark Office.

The full Association insignia, illustrated below, is comprised of the logo and the tagline, "The Scientific Association Dedicated to Analytical Excellence," shown below. The typeface used is Largo. The AOAC tagline is owned by the Association and is registered with the U.S. Patent and Trademark office.

The Scientific Association Dedicated to Analytical Excellence®

Policy

Policy on the use of the Association's name and logo is established by the AOAC Board of Directors as follows:

> "The Board approves and encourages reference to the Association by name, either as AOAC INTERNATIONAL or as AOAC; or reference to our registered trademark, AOAC®, in appropriate settings to describe our programs, products, etc., in scientific literature and other instances so long as the reference is fair, accurate, complete and truthful and does not indicate or imply unauthorized endorsement of any kind.
>
> The insignia (logo) of AOAC INTERNATIONAL is a registered trade and service mark and shall not be reproduced or used by any person or organization other than the Association, its elected and appointed officers, sections, or committees, without the prior written permission of the Association. Those authorized to use the AOAC INTERNATIONAL insignia shall use it only for

the purposes for which permission has been specifically granted.

The name and insignia of the Association shall not be used by any person or organization in any way which indicates, tends to indicate, or implies AOAC official endorsement of any product, service, program, company, organization, event or person, endorsement of which, has not been authorized by the Association, or which suggests that membership in the Association is available to any organization."

The Executive Director, in accordance with the above stated policy, is authorized to process, approve, fix rules, and make available materials containing the Association name and insignia.

It should be noted that neither the Association's name nor its insignia nor part of its insignia may be incorporated into any personal, company, organization, or any other stationery other than that of the Association; nor may any statement be included in the printed portion of such stationery which states or implies that an individual, company, or other organization is a member of the Association.

Instructions

1. Reproduction or use of the Association name or insignia requires prior approval by the Executive Director or his designate.

2. Association insignia should not be altered in any manner without approval of the Executive Director or his designate, except to be enlarged or reduced in their entirety.

3. Artwork for reproducing the Association name or insignia, including those incorporating approved alterations, will be provided on request to those authorized to use them (make such requests to the AOAC Marketing Department). Examples of the types of alterations that would be approved are inclusion of a section name in or the addition of an officer's name and address to the letterhead insignia.

4. When the Association name is used without other text as a heading, it should, when possible, be set in the Largo typeface.

5. Although other colors may be used, AOAC blue, PMS 287, is the preferred color when printing the AOAC insignia, especially in formal and official documents. It is, of course, often necessary and acceptable to reproduce the insignia in black.

6. Do not print one part of the logo or insignia in one color and other parts in another color.

7. The letterhead of AOAC INTERNATIONAL shall not be used by any person or organization other than the Association, elected and appointed officers, staff, sections, or committees; except by special permission.

   Correspondence of AOAC official business should be conducted using AOAC letterhead. However, those authorized to use AOAC letterhead shall use it for official AOAC business only.

   Copies of all correspondence using AOAC letterhead or conducting AOAC official business,

whether on AOAC letterhead or not, must be sent to the appropriate office at AOAC headquarters.

8. AOAC INTERNATIONAL business cards shall not be used by any person or organization other than the Association, its staff, and elected officials, except by special permission.

   Those authorized to use AOAC business cards shall use them for official AOAC business only and shall not represent themselves as having authority to bind the Association beyond that authorized.

Sanctions

1. Upon learning of any violation of the above policy, the Executive Director or a designate will notify the individual or organization that they are in violation of AOAC policy and will ask them to refrain from further misuse of the AOAC name or insignia.

2. If the misuse is by an Individual Member or Sustaining Member of the Association, and the misuse continues after notification, the Board of Directors will take appropriate action.

3. If continued misuse is by a nonmember of the Association or if a member continues misuse in spite of notification and Board action, ultimately, the Association will take legal action to protect its property, legal integrity, reputation, and image.

* * * * * *

Adopted by the AOAC Board of Directors: September 24, 1989
Revised: June 13, 1991; February 26, 1992; March 21, 1995; October 1996

Appendix F: Guidelines for Standard Method Performance Requirements

Contents

Introduction to Standard Method Performance Requirements

Standard method performance requirements (SMPRs) are a unique and novel concept for the analytical methods community. SMPRs are voluntary consensus standards, developed by stakeholders, that prescribe the minimum analytical performance requirements for classes of analytical methods. In the past, analytical methods were evaluated and the results compared to a "gold standard" method, or if a gold standard method did not exist, then reviewers would decide retrospectively if the analytical performance was acceptable. Frequently, method developers concentrated on the process of evaluating the performance parameters of a method, and rarely set acceptance criteria. However, as the *Eurachem Guide* points out: " . . . the judgment of method suitability for its intended use is equally important . . ." (1) to the evaluation process.

International Voluntary Consensus Standards

An SMPR is a form of an international, voluntary consensus standard. A standard is an agreed, repeatable way of doing something that is published as document that contains a technical specification or other precise criteria designed to be used consistently as a rule, guideline, or definition. SMPRs are a *consensus* standards developed by stakeholders in a very controlled process that ensures that users, research organizations, government departments, and consumers work together to create a standard that meets the demands of the analytical community and technology. SMPRs are also *voluntary* standards. AOAC cannot, and does not, impose the use of SMPRs. Users are free to use SMPRs as they see fit. AOAC is very careful to include participants from as many regions of the world as possible so that SMPRs are accepted as *international* standards.

Guidance for Standard Method Performance Requirements

Commonly known as the "SMPR Guidelines." The first version of the SMPR Guidelines were drafted in 2010 in response to the increasing use and popularity of SMPRs as a vehicle to describe the analytical requirements of a method. Several early "acceptance criteria" documents were prepared for publication in late 2009, but the format of the acceptance criteria documents diverged significantly from one another in basic format. AOAC realized that a guidance document was needed to promote uniformity.

An early version of the SMPR Guidelines were used for a project to define the analytical requirements for endocrine disruptors in potable water. The guidelines proved to be extremely useful in guiding the work of the experts and resulted in uniform SMPRs. Subsequent versions of the SMPR Guidelines were used in the Stakeholder Panel for Infant Formula and Adult Nutritionals (SPIFAN) project with very positive results. The SMPR Guidelines are now published for the first time in the *Journal of AOAC INTERNATIONAL* and *Official Methods of Analysis*.

Users of the guidelines are advised that they are: (*1*) a *guidance* document, not a statute that users must conform to; and (*2*) a "living" document that is regularly updated, so users should check the AOAC website for the latest version before using these guidelines.

The SMPR Guidelines are intended to provide basic information for working groups assigned to prepare SMPRs. The guidelines consist of the standard format of an SMPR, followed by a series of informative tables and annexes.

SMPR Format

The general format for an SMPR is provided in *Annex A*.

Each SMPR is identified by a unique SMPR number consisting of the year followed by a sequential identification number (YYYY.XXX). An SMPR number is assigned when the standard is approved. By convention, the SMPR number indicates the year a standard is approved (as opposed to the year the standard is initiated). For example, SMPR 2010.003 indicates the third SMPR adopted in 2010.

The SMPR number is followed by a method name that must include the analyte(s), matrix(es), and analytical technique (unless the SMPR is truly intended to be independent of the analytical technology). The method name may also refer to a "common" name (e.g., "Kjeldahl" method).

The SMPR number and method name are followed by the name of the stakeholder panel or expert review panel that approved the SMPR, and the approval and effective dates.

Information about method requirements is itemized into nine categories: (*1*) intended use; (*2*) applicability; (*3*) analytical technique; (*4*) definitions; (*5*) method performance requirements; (*6*) system suitability; (*7*) reference materials; (*8*) validation guidance; and (*9*) maximum time-to-determination.

An SMPR for qualitative and/or identification methods may include up to three additional annexes: (*1*) inclusivity/selectivity panel; (*2*) exclusivity/cross-reactivity panel; and (*3*) environmental material panels. These annexes not required.

Informative tables.—The SMPR Guidelines contain seven informative tables that represent the distilled knowledge of many years of method evaluation, and are intended as guidance for SMPR working groups. The informative tables are not necessarily AOAC

policy. SMPR working groups are expected to apply their expertise in the development of SMPRs.

Table A1: Performance Requirements. Provides recommended performance parameters to be included into an SMPR. Table A1 is organized by five method classifications: (*1*) main component quantitative methods; (*2*) trace or contaminant quantitative methods; (*3*) main component qualitative methods; (*4*) trace or contaminant quantitative methods; and (*5*) identification methods. The table is designed to accommodate both microbiological and chemical methods. Alternate microbiological/chemical terms are provided for equivalent concepts.

Table A2: Recommended Definitions. Provides definitions for standard terms in the SMPR Guidelines. AOAC relies on *The International Vocabulary of Metrology Basic and General Concepts and Associated Terms* (VIM) and the International Organization for Standadization (ISO) for definition of terms not included in Table A2.

Table A3: Recommendations for Evaluation. Provides general guidance for evaluation of performance parameters. More detailed evaluation guidance can be found in *Appendix D, Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis* (2); *Appendix I, Guidelines for Validation of Biological Threat Agent Methods and/or Procedures* (3); *Appendix K, AOAC Guidelines for Single-Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals* (4); Codex Alimentarius Codex Procedure Manual (5); and ISO Standard 5725-1-1994 (6).

Table A4: Expected Precision (Repeatability) as a Function of Analyte Concentration. The precision of a method is the closeness of agreement between independent test results obtained under stipulated conditions. Precision is usually expressed in terms of imprecision and computed as a relative standard deviation (RSD) of the test results. The imprecision of a method increases as the concentration of the analyte decreases. This table provides target RSDs for a range of analyte concentrations.

Table A5: Expected Recovery as a Function of Analyte Concentration. Recovery is defined as the ratio of the observed mean test result to the true value. The range of the acceptable mean recovery expands as the concentration of the analyte decreases. This table provides target mean recovery ranges for analyte concentrations from 1 ppb to 100%.

Table A6: Predicted Relative Standard Deviation of Reproducibility ($PRSD_R$). This table provides the calculated $PRSD_R$ using the Horwitz formula:

$$PRSD_R = 2C^{-0.15}$$

where C is expressed as a mass fraction.

Table A7: POD and Number of Test Portions. This table provides the calculated probability of detection (POD) for given sample sizes and events (detections). A method developer can use this table to determine the number of analyses required to obtain a specific POD.

Informative annexes.—The SMPR Guidelines contain informative annexes on the topics of classification of methods, POD model, HorRat values, reference materials, and method accuracy and review. As with the informative tables, these annexes are intended to provide guidance and information to the working groups.

Initiation of an SMPR

See Figure 1 for a schematic flowchart diagram of the SMPR development process.

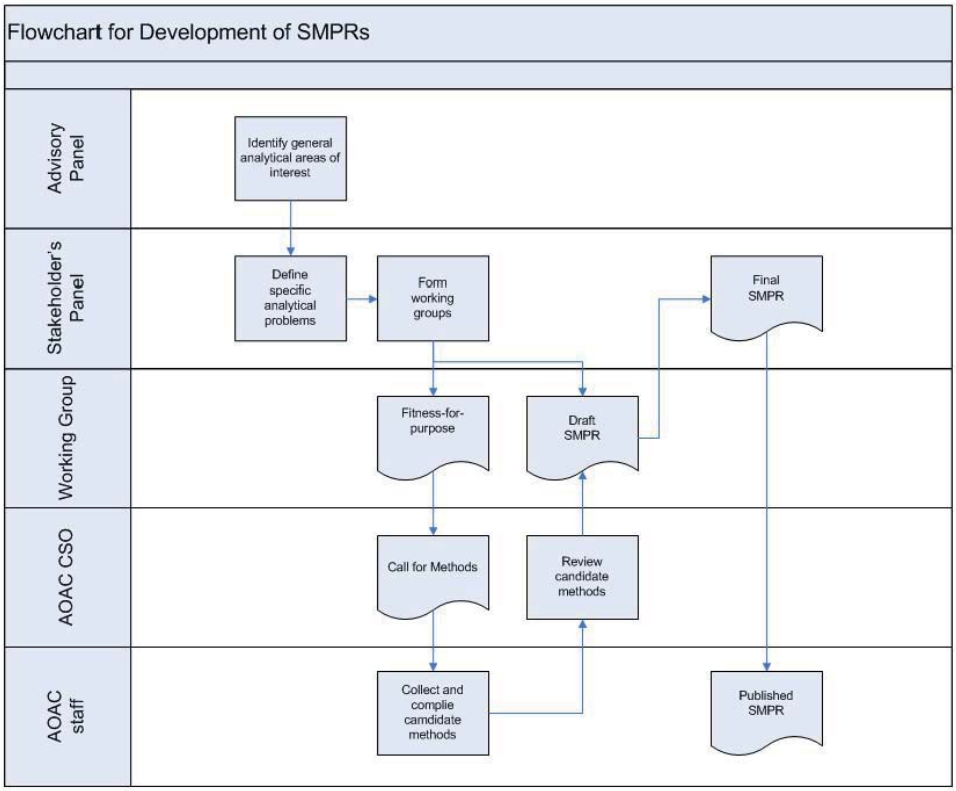


Figure 1. Schematic flowchart diagram of the SMPR development process.

Advisory panels.—Most commonly, an SMPR is created in response to an analytical need identified by an advisory panel. Advisory panels normally consist of sponsors and key stakeholders who have organized to address analytical problems. Usually, the advisory panel identifies general analytical problems, such as the need to update analytical methods for determination of nutrients in infant formula. An advisory panel, with the input of appropriate subject matter experts, also prioritizes the specific analytical problems within the general topic. This panel is critical in planning for the stakeholder panel meeting.

Stakeholder panels.—After an advisory panel has identified a general analytical problem, AOAC announces the standards development activity, identifies stakeholders, and organizes a stakeholder panel. Membership on a stakeholder panel is open to anyone materially affected by the proposed standard. AOAC recruits scientists to participate on stakeholder panels on the basis of their expertise with the analytical problem identified by the advisory panel. Experts are recruited from academia, government, nongovernmental organizations (such as ISO), industry, contract research organizations, method developers, and instrument/equipment manufacturers. AOAC employs a representative voting panel model to ensure balance with regards to stakeholder perspective, and to ensure that no particular stakeholder perspective dominates the proceedings of the stakeholder panel. All stakeholder candidates are reviewed by the AOAC Chief Scientific Officer (CSO) for relevant qualifications, and again by the Official Methods Board to ensure that the stakeholder panel is balanced and all stakeholders are fairly represented.

Stakeholder panels are extremely important as they serve several functions: (*1*) identify specific analytical topics within the general analytical problem described by the advisory panel; (*2*) form working groups to address the specific analytical topics; (*3*) identify additional subject matter experts needed for the working groups; (*4*) provide oversight of the SMPR development; and (*5*) formally adopt SMPRs originally drafted by working groups.

Working groups.—Working groups are formed by the stakeholder panel when a specific analytical topic has been identified. The primary purpose of a working group is to draft an SMPR. Working groups may also be formed to make general recommendations, such as developing a common definition to be used by multiple working groups. For example, SPIFAN formed a working group to create a definition for "infant formula" that could be shared and used by all of the SPIFAN working groups.

The process of drafting an SMPR usually requires several months, and several meetings and conference calls. An SMPR drafted by a working group is presented to a stakeholder panel. A stakeholder panel may revise, amend, or adopt a proposed SMPR on behalf of AOAC.

Fitness-for-Purpose Statement and Call for Methods

One of the first steps in organizing a project is creating a fitness-for-purpose statement. In AOAC, the fitness-for-purpose statement is a very general description of the methods needed. It is the responsibility of a working group chair to draft a fitness-for-purpose statement. A working group chair is also asked to prepare a presentation with background information about the analyte, matrix, and the nature of the analytical problem. A working group chair presents the background information and proposes a draft fitness-for-purpose statement to the presiding stakeholder panel. The stakeholder panel is asked to endorse the fitness-for-purpose statement.

The AOAC CSO prepares a call for methods based on the stakeholder panel-approved fitness-for-purpose statement. The call for methods is posted on the AOAC website and/or e-mailed to the AOAC membership and other known interested parties. AOAC staff collects and compiles candidate methods submitted in response to the call for methods. The CSO reviews and categorizes the methods.

Creating an SMPR

Starting the process of developing an SMPR can be a daunting challenge. In fact, drafting an SMPR should be a daunting challenge because the advisory panel has specifically identified an analytical problem that has yet to be resolved. Completing an SMPR can be a very rewarding experience because working group members will have worked with their colleagues through a tangle of problems and reached a consensus where before there were only questions.

It is advisable to have some representative candidate methods available for reference when a working group starts to develop an SMPR. These methods may have been submitted in response to the call for methods, or may be known to a working group member. In any case, whatever the origin of the method, candidate methods may assist working group members to determine reasonable performance requirements to be specified in the SMPR. The performance capabilities of exisiting analytical methodologies is a common question facing a working group.

Normally, a working chair and/or the AOAC CSO prepares a draft SMPR. A draft SMPR greatly facilitates the process and provides the working group with a structure from which to work.

Working group members are advised to first consider the "intended use" and "maximum time-to-determination" sections as this will greatly affect expectations for candidate methods. For example, methods intended to be used for surveillance probably need to be quick but do not require a great deal of precision, and false-positive results might be more tolerable. Whereas methods intended to be used for dispute resolution will require better accuracy, precision, and reproducibility, but time to determination is not as important.

Once a working group has agreed on the intended use of candidate methods, then it can begin to define the applicability of candidate methods. The applicability section of the SMPR is one of the most important, and sometimes most difficult, sections of the SMPR. The analyte(s) and matrixes must be explicitly identified. For chemical analytes, International Union of Pure and Applied Chemistry (IUPAC) nomenclature and/or Chemical Abstracts Service (CAS) registry numbers should be specified. Matrixes should be clearly identified including the form of the matrix such as raw, cooked, tablets, powders, etc. The nature of the matrix may affect the specific analyte. It may be advantageous to fully identify and describe the matrix before determining the specific analyte(s). It is not uncommon for working groups to revise the initial definition of the analyte(s) after the matrix(es) has been better defined.

Table 1. Example of method performance table for a single analyte

Analytical range	7.0–382.6 µg/mL	
Limit of quantitation (LOQ)	≤7.0 µg/mL	
Repeatability (RSD_r)	<10 µg/mL	≤8%
	≥10 µg/mL	≤6%

Table 2. Example of method performance table for multiple analytes

	Analyte 1		Analyte 2		Analyte 3	
Analytical range	10–20 µg/mL		100–200 µg/mL		200–500 µg/mL	
Limit of quantitation (LOQ)	≤10 µg/mL		≤100 µg/mL		≤200 µg/mL	
Repeatability (RSD_r)	<10 µg/mL	≤8%	<10 µg/mL	≤8%	<200 µg/mL	≤10%
	≥10 µg/mL	≤6%	≥10 µg/mL	≤6%	≥200 µg/mL	≤8%

For projects with multiple analytes, for example, vitamins A, D, E, and K in infant formula, it may be useful to organize a separate working group to fully describe the matrix(es) so that a common description of the matrix(es) can be applied to all of the analytes.

For single analyte SMPRs, it is most common to organize the method performance requirements into a table with 2–3 columns as illustrated in Table 1. For multiple analyte SMPRs, it is often convenient to present the requirements in an expanded table with analytes forming additional columns as illustrated in Table 2.

Once the intended use, analytical techniques, and method performance requirements have been determined, then a working group can proceed to consider the quality control parameters, such as the minimum validation requirements, system suitability procedures, and reference materials (if available). It is not uncommon that an appropriate reference material is not available. *Annex F* of the SMPR Guidelines provides comprehensive guidance for the development and use of in-house reference materials.

Most working groups are able to prepare a consensus SMPR in about 3 months.

Open Comment Period

Once a working group has produced a draft standard, AOAC opens a comment period for the standard. The comment period provides an opportunity for other stakeholders to state their perspective on the draft SMPR. All collected comments are reviewed by the AOAC CSO and the working group chair, and the comments are reconciled. If there are significant changes required to the draft standard as a result of the comments, the working group is convened to discuss and any unresolved issues will be presented for discussion at the stakeholder panel meeting.

Submission of Draft SMPRs to the Stakeholder Panel

Stakeholder panels meet several times a year at various locations. The working group chair (or designee) presents a draft SMPR to the stakeholder panel for review and discussion. A working group chair is expected to be able to explain the conclusions of the working group, discuss comments received, and to answer questions from the stakeholder panel. The members of the stakeholder panel may revise, amend, approve, or defer a decision on the proposed SMPR. A super majority of 2/3 or more of those voting is required to adopt an SMPR as an AOAC voluntary consensus standard.

Publication

Adopted SMPRs are prepared for publication by AOAC staff, and are published in the *Journal of AOAC INTERNATIONAL* and in the AOAC *Official Methods of Analysis*℠ compendium. Often, the AOAC CSO and working group chair prepare a companion article to introduce an SMPR and describe the analytical issues considered and resolved by the SMPR. An SMPR is usually published within 6 months of adoption.

Conclusion

SMPRs are a unique and novel concept for the analytical methods community. SMPRs are voluntary, consensus standards developed by stakeholders that prescribe the minimum analytical performance requirements for classes of analytical methods. The SMPR Guidelines provide a structure for working groups to use as they develop an SMPR. The guidelines have been employed in several AOAC projects and have been proven to be very useful. The guidelines are not a statute that users must conform to; they are a "living" document that is regularly updated, so users should check the AOAC website for the latest version before using the guidelines.

References

(1) Eurachem, *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*, *Validation*, http://www.eurachem.org/guides/pdf/valid.pdf, posted December 1998, accessed March 2012
(2) *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis* (2012) *Official Methods of Analysis*, *Appendix D*, AOAC INTERNATIONAL, Gaithersburg, MD
(3) *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures* (2012) *Official Methods of Analysis*, 19th Ed., *Appendix I, Calculation of CPOD and dCPOD Values from Qualitative Method Collaborative Study Data*, AOAC INTERNATIONAL, Gaithersburg, MD
(4) *AOAC Guidelines for Single-Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals* (2012) *Official Methods of Analysis*, 19th Ed., *Appendix K*, AOAC INTERNATIONAL, Gaithersburg, MD
(5) Codex Alimentarius Codex Procedure Manual
(6) International Organization for Standardization, Geneva, Switzlerland

ANNEX A
Format of a
Standard Method Performance Requirement

AOAC SMPR YYYY.XXX
(YYYY = Year; XXX = sequential identification number)

Method Name: Must include the analyte(s), matrix(es), and analytical technique [unless the standard method performance requirement (SMPR) is truly intended to be independent of the analytical technology]. The method name may refer to a "common" name (e.g., "Kjeldahl" method).

Approved By: Name of stakeholder panel or expert review panel

Final Version Date: Date

Effective Date: Date

1. Intended Use: Additional information about the method and conditions for use.

2. Applicability: List matrixes if more than one. Provide details on matrix such as specific species for biological analytes, or International Union of Pure and Applied Chemistry (IUPAC) nomenclature and Chemical Abstracts Service (CAS) registry number for chemical analytes. Specify the form of the matrix such as raw, cooked, tablets, powders, etc.

3. Analytical Technique: Provide a detailed description of the analytical technique if the SMPR is to apply to a specific analytical technique; or state that the SMPR applies to any method that meets the method performance requirements.

4. Definitions: List and define terms used in the performance parameter table (*see* Table A2 for list of standard terms).

5. Method Performance Requirements: List the performance parameters and acceptance criteria appropriate for each method/analyte/matrix. *See* Table A1 for appropriate performance requirements.

If more than one analyte/matrix, and if acceptance criteria differ for analyte/matrix combinations then organize a table listing each analyte/matrix combination and its minimum acceptance criteria for each performance criteria.

6. System Suitability Tests and/or Analytical Quality Control: Describe minimum system controls and QC procedures.

7. Reference Material(s): Identify the appropriate reference materials if they exist, or state that reference materials are not available. Refer to *Annex E* (*AOAC Method Accuracy Review*) for instructions on the use of reference materials in evaluations.

8. Validation Guidance: Recommendations for type of evaluation or validation program such as single-laboratory validation (SLV), *Official Methods of Analysis*℠ (OMA), or *Performance Tested Methods*℠ (PTM).

9. Maximum Time-to-Determination: Maximum allowable time to complete an analysis starting from the test portion preparation to final determination or measurement.

Annex I: Inclusivity/Selectivity Panel. Recommended for qualitative and identification method SMPRs.

Annex II: Exclusivity/Cross-Reactivity Panel. Recommended for qualitative and identification method SMPRs.

Annex III: Environmental Materials Panel. Recommended for qualitative and identification method SMPRs.

Table A1. Performance requirements

Classifications of methods[a]				
Quantitative method		Qualitative method		
Main component[b]	Trace or contaminant[c]	Main component[b]	Trace or contaminant[c]	Identification method
Parameter				
Single-laboratory validation				
Applicable range	Applicable range	Inclusivity/selectivity	Inclusivity/selectivity	Inclusivity/selectivity
Bias[d]	Bias[d]	Exclusivity/cross-reactivity	Exclusivity/cross-reactivity	Exclusivity/cross-reactivity
Precision	Precision	Environmental interference	Environmental interference	Environmental interference
Recovery	Recovery	Laboratory variance	Laboratory variance	
Limit of quantitation (LOQ)	LOQ			
		Probability of detection (POD)[e]	POD at AMDL[f]	Probability of identification (POI)
Reproducibility				
RSD_R or target measurement uncertainty	RSD_R or target measurement uncertainty	POD (0)	POD (0)	POI (c)
		POD (c)	POD (c)	
		Laboratory POD[g]	Laboratory POD[g]	Laboratory POI

[a] *See Annex B* for additional information on classification of methods.

[b] ≥100 g/kg.

[c] <100 g/kg.

[d] If a reference material is available.

[e] At a critical level.

[f] AMDL = Acceptable minimum detection level.

[g] LPOD = CPOD.

Table A2. Recommended definitions

Bias	Difference between the expectation of the test results and an accepted reference value. Bias is the total systematic error as contrasted to random error. There may be one or more systematic error components contributing to the bias.
Environmental interference	Ability of the assay to detect target organism in the presence of environmental substances and to be free of cross reaction from environmental substances.
Exclusivity	Strains or isolates or variants of the target agent(s) that the method must not detect.
Inclusivity	Strains or isolates or variants of the target agent(s) that the method can detect.
Laboratory probability of detection (POD)	Overall fractional response (mean POD = CPOD) for the method calculated from the pooled POD_j responses of the individual laboratories (j = 1, 2, ..., L).[a] *See Annex C*.
Limit of quantitation (LOQ)	Minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.
POD (0)	Probability of the method giving a (+) response when the sample is truly without analyte.
POD (c)	Probability of the method giving a (–) response when the sample is truly without analyte.
POD	Proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. Consult *Annex C* for a full explanation.
Probability of identification (POI)	Expected or observed fraction of test portions at a given concentration that gives positive result when tested at a given concentration. Consult *Probability of Identification (POI): A Statistical Model for the Validation of Qualitative Botanical Identification Methods*.[c]
Precision (repeatability)	Closeness of agreement between independent test results obtained under stipulated conditions. The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation of the test results.[d]
Recovery	Fraction or percentage of the analyte that is recovered when the test sample is analyzed using the entire method. There are two types of recovery: (*1*) Total recovery based on recovery of the native plus added analyte, and (*2*) marginal recovery based only on the added analyte (the native analyte is subtracted from both the numerator and denominator).[e]
Repeatability	Precision under repeatability conditions.
Repeatability conditions	Conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time.
Reproducibility	Precision under reproducibility conditions.
Reproducibility conditions	Conditions where independent test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment.
Relative standard deviation (RSD)	$RSD = s_i \times 100/\bar{x}$
Standard deviation (s_i)	$s_i = [\Sigma(x_i - \bar{x})^2/n]^{0.5}$

[a] *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures* (*Calculation of CPOD and dCPOD Values from Qualitative Method Collaborative Study Data*), *J. AOAC Int*. **94**, 1359(2011) and *Official Methods of Analysis of AOAC INTERNATIONAL* (2012) 19th Ed., *Appendix I*.

[b] *International Vocabulary of Metrology (VIM)—Basic and General Concepts and Associated Terms* (2008) JCGM 200:2008, Joint Committee for Guides in Metrology (JCGM), www.bipm.org

[c] LaBudde, R.A., & Harnly, J.M. (2012) *J. AOAC Int*. **95**, 273–285.

[d] ISO 5725-1-1994.

[e] *Official Methods of Analysis* (2012) *Appendix D* (*Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis*), AOAC INTERNATIONAL, Gaithersburg, MD.

Table A3. Recommendations for evaluation

Bias (if a reference material is available)	A minimum of five replicate analyses of a Certified Reference Material.[a]
Environmental interference	Analyze test portions containing a specified concentration of one environmental materials panel member. Materials may be pooled. Consult with AOAC statistician.
Exclusivity/cross-reactivity	Analyze one test portion containing a specified concentration of one exclusivity panel member. More replicates can be used. Consult with AOAC statistician.
Inclusivity/selectivity	Analyze one test portion containing a specified concentration of one inclusivity panel member. More replicates can be used. Consult with AOAC statistician.
Limit of quantitation (LOQ)	Estimate the LOQ = average (blank) + 10 × s_0 (blank). Measure blank samples with analyte at the estimated LOQ. Calculate the mean average and standard deviation of the results. Guidance[b]: For ML ≥ 100 ppm (0.1 mg/kg): LOD = ML × 1/5. For ML < 100 ppm (0.1 mg/kg): LOD = ML × 2/5.
Measurement uncertainty	Use ISO 21748: *Guidance for the use of repeatability, reproducibility, and trueness estimates in measurement uncertainty estimation to analyze data collected for bias, repeatability, and intermediate precision to estimate measurement uncertainty*.
POD(0)	Use data from collaborative study.
POD (c)	
Repeatability	Prepare and homogenize three unknown samples at different concentrations to represent the full, claimed range of the method. Analyze each unknown sample by the candidate method seven times, beginning each analysis from weighing out the test portion through to final result with no additional replication (unless stated to do so in the method). All of the analyses for one unknown sample should be performed within as short a period of time as is allowed by the method. The second and third unknowns may be analyzed in another short time period. Repeat for each claimed matrix.
Probability of detection (POD)	Determine the desired POD at a critical concentration. Consult with Table A7 to determine the number of test portions required to demonstrate the desired POD.
Probability of identification (POI)	Consult *Probability of Identification (POI): A Statistical Model for the Validation of Qualitative Botanical Identification Methods*[c].
Recovery	Determined from spiked blanks or samples with at least seven independent analyses per concentration level at a minimum of three concentration levels covering the analytical range. Independent means at least at different times. If no confirmed (natural) blank is available, the average inherent (naturally containing) level of the analyte should be determined on at least seven independent replicates. Marginal % recovery = $(C_f - C_u) \times 100/C_A$ Total % recovery = $100(C_f)/(C_u + C_A)$ where C_f = concentration of fortified samples, C_u = concentration of unfortified samples, and C_A = concentration of analyte added to the test sample.[d] Usually total recovery is used unless the native analyte is present in amounts greater than about 10% of the amount added, in which case use the method of addition.[e]
Reproducibility (collaborative or interlaboratory study)	Quantitative methods: Recruit 10–12 collaborators; must have eight valid data sets; two blind duplicate replicates at five concentrations for each analyte/matrix combination to each collaborator.
	Qualitative methods: Recruit 12–15 collaborators; must have 10 valid data sets; six replicates at five concentrations for each analyte/matrix combination to each collaborator.

[a] *Guidance for Industry for Bioanalytical Method Validation* (May 2001) U.S. Department of Health and Human Services, U.S. Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM).

[b] Codex Alimentarius Codex Procedure Manual.

[c] LaBudde, R.A., & Harnly, J.M. (2012) *J. AOAC Int.* **95**, 273–285.

[d] *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis* (2012) *Official Methods of Analysis*, 19th Ed., *Appendix D,* AOAC INTERNATIONAL, Gaithersburg, MD.

[e] *AOAC Guidelines for Single-Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals* (2012) *Official Methods of Analysis*, 19th Ed., *Appendix K*, AOAC INTERNATIONAL, Gaithersburg, MD.

Table A4. Expected precision (repeatability) as a function of analyte concentration[a]

Analyte, %	Analyte ratio	Unit	RSD, %
100	1	100%	1.3
10	10^{-1}	10%	1.9
1	10^{-2}	1%	2.7
0.01	10^{-3}	0.1%	3.7
0.001	10^{-4}	100 ppm (mg/kg)	5.3
0.0001	10^{-5}	10 ppm (mg/kg)	7.3
0.00001	10^{-6}	1 ppm (mg/kg)	11
0.000001	10^{-7}	100 ppb (µg/kg)	15
0.0000001	10^{-8}	10 ppb (µg/kg)	21
0.00000001	10^{-9}	1 ppb (µg/kg)	30

[a] Table excerpted from *AOAC Peer-Verified Methods Program, Manual on Policies and Procedures* (1998) AOAC INTERNATIONAL, Gaithersburg, MD.

The precision of a method is the closeness of agreement between independent test results obtained under stipulated conditions. Precision is usually expressed in terms of imprecision and computed as a relative standard deviation of the test results. The imprecision of a method increases as the concentration of the analyte decreases. This table provides targets RSDs for a range of analyte concentrations.

Table A5. Expected recovery as a function of analyte concentration[a]

Analyte, %	Analyte ratio	Unit	Mean recovery, %
100	1	100%	98–102
10	10^{-1}	10%	98–102
1	10^{-2}	1%	97–103
0.01	10^{-3}	0.1%	95–105
0.001	10^{-4}	100 ppm	90–107
0.0001	10^{-5}	10 ppm	80–110
0.00001	10^{-6}	1 ppm	80–110
0.000001	10^{-7}	100 ppb	80–110
0.0000001	10^{-8}	10 ppb	60–115
0.00000001	10^{-9}	1 ppb	40–120

[a] Table excerpted from *AOAC Peer-Verified Methods Program, Manual on Policies and Procedures* (1998) AOAC INTERNATIONAL, Gaithersburg, MD.

Recovery is defined as the ratio of the observed mean test result to the true value. The range of the acceptable mean recovery expands as the concentration of the analyte decreases. This table provides target mean recovery ranges for analyte concentrations from 100% to 1 ppb.

Table A6. Predicted relative standard deviation of reproducibility ($PRSD_R$)[a]

Concentration (C)	Mass fraction (C)	$PRSD_R$, %
100%	1.0	2
1%	0.01	4
0.01%	0.0001	8
1 ppm	0.000001	16
10 ppb	0.00000001	32
1 ppb	0.000000001	45

[a] Table excerpted from *Definitions and Calculations of HorRat Values from Intralaboratory Data*, HorRat for SLV.doc, 2004-01-18, AOAC INTERNATIONAL, Gaithersburg, MD.

Predicted relative standard deviation = $PRSD_R$. Reproducibility relative standard deviation calculated from the Horwitz formula:

$$PRSD_R = 2C^{-0.15}, \text{ where C is expressed as a mass fraction}$$

This table provides the calculated $PRSD_R$ for a range of concentrations. *See Annex D* for additional information.

Table A7. POD and number of test portions[a,b]

	Sample size required for proportion						
Assume	1. Binary outcome (occur/not occur). 2. Constant probability rho of event occurring. 3. Independent trials (e.g., simple random sample). 4. Fixed number of trials (*N*)						
Inference	95% Confidence interval lies entirely at or above specified minimum rho						
Desired	Sample size *N* needed						
Minimum probability rho, %	Sample size (*N*)	Minimum No. events (*x*)	Maximum No. nonevents (*y*)	1-Sided lower confidence limit on rho[c], %	Expected lower confidence limit on rho, %	Expected upper confidence limit on rho, %	Effective AOQL[d] rho, %
50	3	3	0	52.6	43.8	100.0	71.9
50	10	8	2	54.1	49.0	94.3	71.7
50	20	14	6	51.6	48.1	85.5	66.8
50	40	26	14	52.0	49.5	77.9	63.7
50	80	48	32	50.8	49.0	70.0	59.5
55	4	4	0	59.7	51.0	100.0	75.5
55	10	9	1	65.2	59.6	100.0	79.8
55	20	15	5	56.8	53.1	88.8	71.0
55	40	28	12	57.1	54.6	81.9	68.2
55	80	52	28	55.9	54.1	74.5	64.3
60	5	5	0	64.9	56.5	100.0	78.3
60	10	9	1	65.2	59.6	100.0	79.8
60	20	16	4	62.2	58.4	91.9	75.2
60	40	30	10	62.4	59.8	85.8	72.8
60	80	56	24	61.0	59.2	78.9	69.1
65	6	6	0	68.9	61.0	100.0	80.5
65	10	9	1	65.2	59.6	100.0	79.8
65	20	17	3	67.8	64.0	94.8	79.4
65	40	31	9	65.1	62.5	87.7	75.1
65	80	59	21	65.0	63.2	82.1	72.7
70	7	7	0	72.1	64.6	100.0	82.3
70	10	10	0	78.7	72.2	100.0	86.1
70	20	18	2	73.8	69.9	97.2	83.6
70	40	33	7	70.7	68.0	91.3	79.7
70	80	63	17	70.4	68.6	86.3	77.4
75	9	9	0	76.9	70.1	100.0	85.0
75	10	10	0	78.7	72.2	100.0	86.1
75	20	19	1	80.4	76.4	100.0	88.2
75	40	35	5	76.5	73.9	94.5	84.2
75	80	67	13	75.9	74.2	90.3	82.2
80	11	11	0	80.3	74.1	100.0	87.1
80	20	19	1	80.4	76.4	100.0	88.2
80	40	37	3	82.7	80.1	97.4	88.8
80	80	70	10	80.2	78.5	93.1	85.8
85	20	20	0	88.1	83.9	100.0	91.9
85	40	38	2	86.0	83.5	98.6	91.1
85	80	74	6	86.1	84.6	96.5	90.6
90	40	40	0	93.7	91.2	100.0	95.6
90	60	58	2	90.4	88.6	99.1	93.9
90	80	77	3	91.0	89.5	98.7	94.1
95	60	60	0	95.7	94.0	100.0	97.0
95	80	80	0	96.7	95.4	100.0	97.7
95	90	89	1	95.2	94.0	100.0	97.0
95	96	95	1	95.5	94.3	100.0	97.2
98	130	130	0	98.0	97.1	100.0	98.6
98	240	239	1	98.2	97.7	100.0	98.8
99	280	280	0	99.0	98.6	100.0	99.3
99	480	479	1	99.1	98.8	100.0	99.4

[a] Table excerpted from Technical Report TR308, *Sampling plans to verify the proportion of an event exceeds or falls below a specified value*, LaBudde, R. (June 4, 2010) (not published). The table was produced as part of an informative report for the Working Group for Validation of Identity Methods for Botanical Raw Materials commissioned by the AOAC INTERNATIONAL Presidential Task Force on Dietary Supplements. The project was funded by the Office of Dietary Supplements, National Institutes of Health.

[b]

[c] Based on modified Wilson score 1-sided confidence interval.

[d] AOQL = Average outgoing quality level.

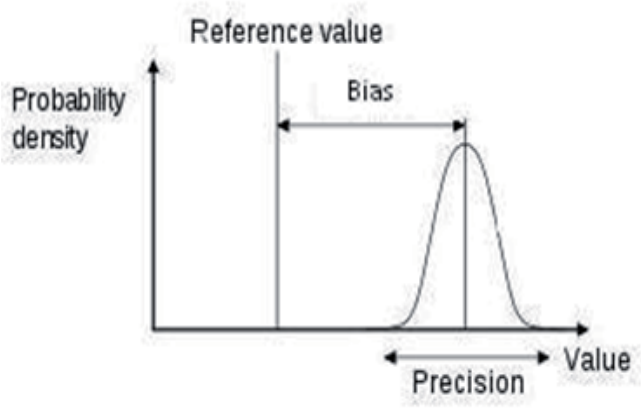


Figure A1. Relationship between precision versus bias (trueness). Trueness is reported as bias. Bias is defined as the difference between the test results and an accepted reference value.

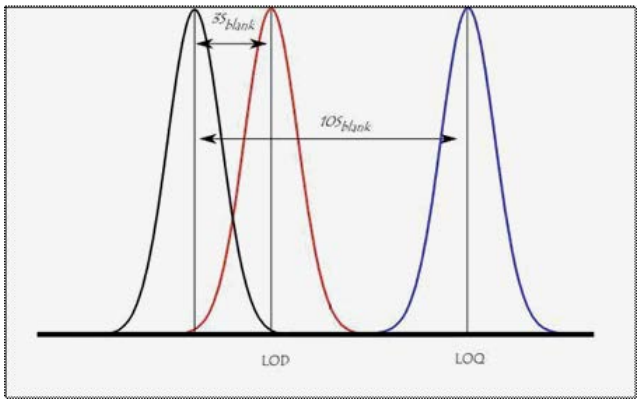


Figure A2. Relationship between LOD and LOQ. LOD is defined as the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit. LOQ is the level above which quantitative results may be obtained with a stated degree of confidence.

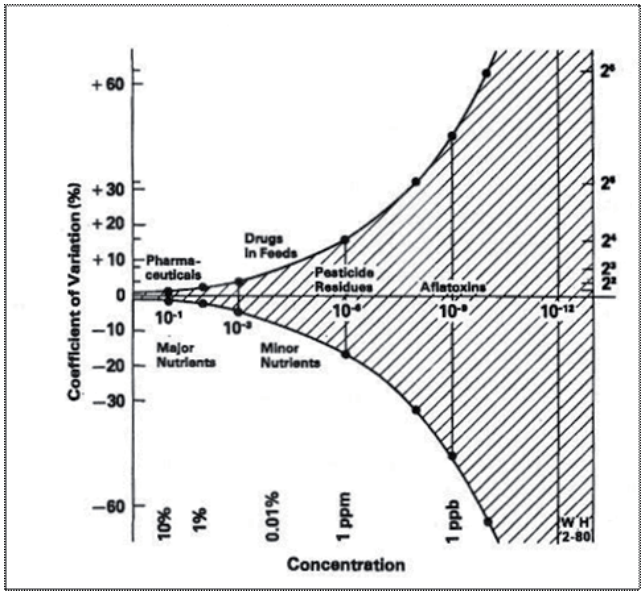


Figure A3. Horwitz Curve, illustrating the exponential increase in the coefficient of variation as the concentration of the analyte decreases [*J. AOAC Int*. 89, 1095(2006)].

ANNEX B
Classification of Methods

The following guidance may be used to determine which performance parameters in Table A1 apply to different classifications of methods. AOAC INTERNATIONAL does not recognize the term "semiquantitative" as a method classification. Methods that have been self-identified as semiquantitative will be classified into one of the following five types:

Type I: Quantitative Methods

Characteristics: Generates a continuous number as a result.

Recommendation: Use performance requirements specified for quantitative method (main or trace component). Use recovery range and maximum precision variation in Tables A4 and A5.

In some cases and for some purposes, methods with less accuracy and precision than recommended in Tables A4 and A5 may be acceptable. Method developers should consult with the appropriate method committee to determine if the recommendations in Tables A4 and A5 do or do not apply to their method.

Type II: Methods that Report Ranges

Characteristics: Generates a "range" indicator such as 0, low, moderate, and high.

Recommendation: Use performance requirements specified for qualitative methods (main component). Specify a range of POD for each range "range" indicator.

Type III: Methods with Cutoff Values

Characteristics: Method may generate a continuous number as an interim result (such as a CT value for a PCR method), which is not reported but converted to a qualitative result (presence/ absence) with the use of a cutoff value.

Recommendation: Use performance requirements specified for qualitative methods.

Type IV: Qualitative Methods

Characteristics: Method of analysis whose response is either the presence or absence of the analyte detected either directly or indirectly in a specified test portion.

Recommendation: Use performance requirements specified for qualitative methods.

Type V: Identification Methods

Characteristics: Method of analysis whose purpose is to determine the identity of an analyte.

Recommendation: Use performance requirements specified for identification methods.

ANNEX C
Understanding the POD Model

Excerpted from AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures, J. AOAC Int. ***94****, 1359(2011) and Official Methods of Analysis of AOAC INTERNATIONAL (2012) 19th Ed., Appendix I.*

The Probability of Detection (POD) model is a way of characterizing the performance of a qualitative (binary) method. A binary qualitative method is one that gives a result as one of two possible outcomes, either positive or negative, presence/absence, or +/–.

The single parameter of interest is the POD, which is defined as the probability at a given concentration of obtaining a positive response by the detection method. POD is assumed to be dependent on concentration, and generally, the probability of a positive response will increase as concentration increases.

For example, at very low concentration, the expectation is that the method will not be sensitive to the analyte, and at very high concentration, a high probability of obtaining a positive response is desired. The goal of method validation is to characterize how method response transitions from low concentration/low response to high concentration/high response.

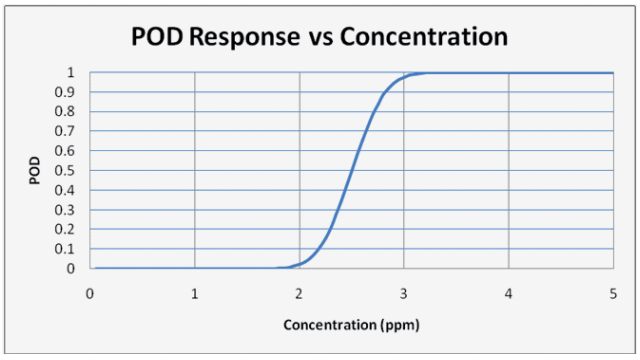


Figure C1. Theoretical POD curve for a qualitative detection method.

POD is always considered to be dependent upon analyte concentration. The POD curve is a graphical representation of method performance, where the probability is plotted as a function of concentration (*see*, for example, Figure C1).

The POD model is designed to allow an objective description of method response without consideration to an a priori expectation of the probabilities at given concentrations. The model is general enough to allow comparisons to any theoretical probability function.

The POD model is also designed to allow for an independent description of method response without consideration to the response of a reference method. The model is general enough to allow for comparisons between reference and candidate method responses, if desired.

Older validation models have used the terms "sensitivity," "specificity," "false positive," and "false negative" to describe method performance. The POD model incorporates all of the performance concepts of these systems into a single parameter, POD.

For example, false positive has been defined by some models as the probability of a positive response, given the sample is truly negative (concentration = 0). The equivalent point on the POD curve for this performance characteristic is the value of the curve at Conc = 0.

Similarly, false negative has sometimes been defined as the probability of a negative response when the sample is truly positive (concentration >0). In the POD curve, this would always be specific to a given sample concentration, but would be represented as the distance from the POD curve to the POD = 1 horizontal top axis at all concentrations except C = 0.

The POD model incorporates all these method characteristics into a single parameter, which is always assumed to vary by concentration. In other models, the terms "false positive," "false negative," "sensitivity," and "specificity" have been defined in a variety of ways, usually not conditional on concentration. For these reasons, these terms are obsolete under this model (*see* Table C1).

The terms "sensitivity," "specificity," "false positive," and "false negative" are obsolete under the POD model (*see* Figure C2).

Table C1. Terminology

Traditional terminology	Concept	POD equivalent	Comment
False positive	Probability of the method giving a (+) response when the sample is truly without analyte	POD(0) POD at conc = 0	POD curve value at conc = 0; "*Y*-intercept" of the POD curve
Specificity	Probability of the method giving a (-) response when the sample is truly without analyte	1-POD(0)	Distance along the POD axis from POD = 1 to the POD curve value
False negative (at a given concentration)	Probability of a (–) response at a given concentration	1-POD(c)	Distance from the POD curve to the POD = 1 "top axis" in the vertical direction
Sensitivity (at a given concentration)	Probability of a (+) response at a given concentration	POD(c)	Value of the POD curve at any given concentration
True negative	A sample that contains no analyte	C = 0	Point on concentration axis where c = 0
True positive	A sample that contains analyte at some positive concentration	C > 0	Range of concentration where c > 0

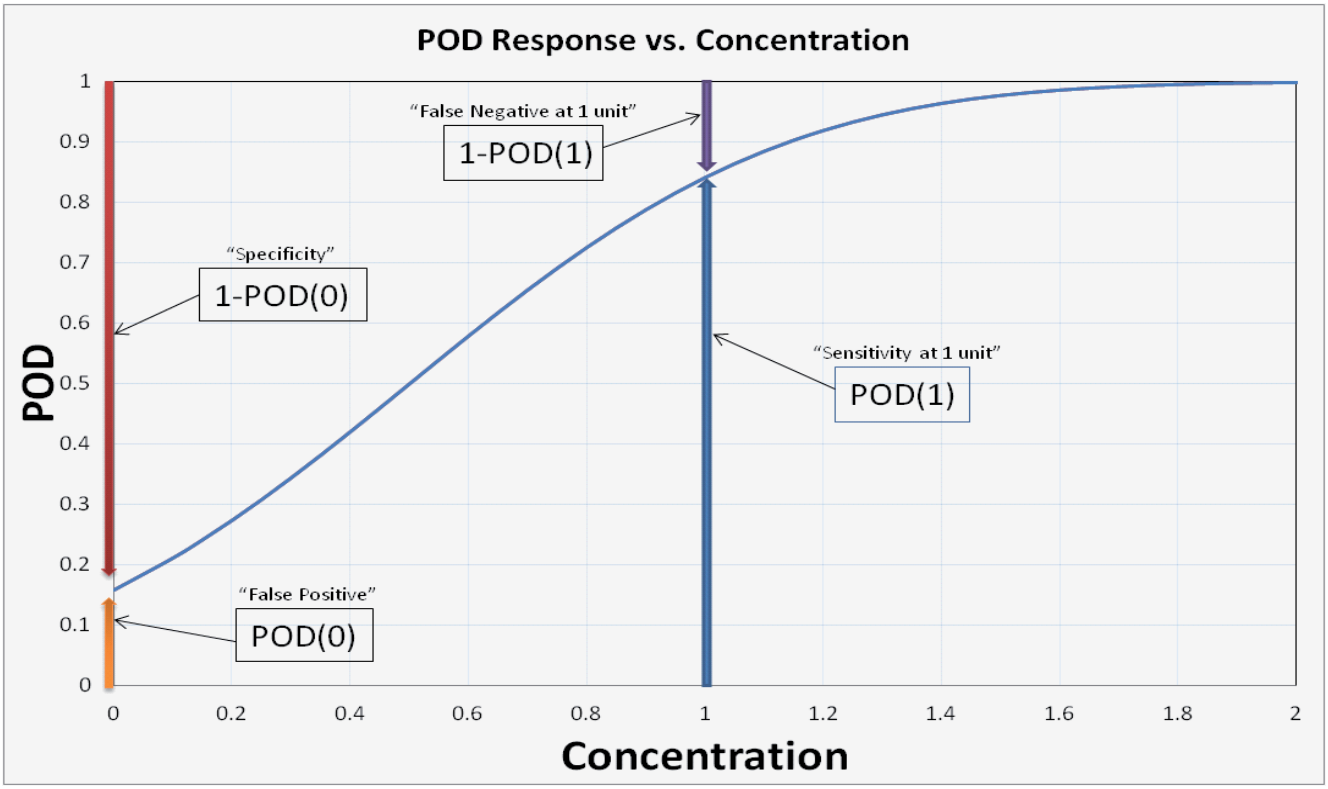


Figure C2. Comparison of POD model terminology to other obsolete terms.

ANNEX D
Definitions and Calculations of HorRat Values from Intralaboratory Data

Excerpted from Definitions and Calculations of HorRat Values from Intralaboratory Data, AOAC INTERNATIONAL, HorRat for SLV.doc, 2004-01-18.

1. Definitions

1.1 Replicate Data

Data developed under common conditions in the same laboratory: simultaneous performance, or, if necessary to obtain sufficient values, same series, same analyst, same day. Such data provides "repeatability statistical parameters."

1.2 Pooled Data

Replicate data developed in the same laboratory under different conditions but considered sufficiently similar that, for the purpose of statistical analysis, they may be considered together. These may include different runs, different instruments, different analysts, and different days.

1.3 Average

0 = Sum of the individual values, x_i, divided by the number of individual values, *n*.

$$0 = (\Sigma\, x_i)/n$$

1.4 Standard Deviation

$$s_i = [\Sigma(x_i - (\bar{x})^2/n]^{0.5}$$

1.5 Relative Standard Deviation

$$RSD = s_i \times 100/\bar{x}$$

1.5.1 Repeatability Relative Standard Deviation [RSD(r) or RSD_r]

The relative standard deviation calculated from within-laboratory data.

1.5.2 Reproducibility Relative Standard Deviation [RSD(R) or RSD_R]

The relative standard deviation calculated from among-laboratory data.

Table D1. Predicted relative standard deviations

Concentration (C)	Mass fraction (C)	$PRSD_R$, %
100%	1.0	2
1%	0.01	4
0.01%	0.0001	8
1 ppm	0.000001	16
10 ppb	0.00000001	32
1 ppb	0.000000001	45

1.6 Mass Fraction

Concentration, C, expressed as a decimal fraction. For calculating and reporting statistical parameters, data may be expressed in any convenient units (e.g., %, ppm, ppb, mg/g, µg/g; µg/kg; µg/L, µg/µL, etc.). For reporting HorRat values, data must be reported as a mass fraction where the units of the numerator and denominator are the same: e.g., for 100% (pure materials), the mass fraction C = 1.00; for 1 µg/g (ppm), C = 0.000001 = (E-6). *See* Table D1 for other examples.

1.7 Predicted Relative Standard Deviation [PRSD(R) or $PRSD_R$]

The reproducibility relative standard deviation calculated from the Horwitz formula:

$$PRSD(R) = 2C^{-0.15}$$

where C is expressed as a mass fraction. *See* Table D1.

In spreadsheet notation: PRSD(R) = 2 * C ^(–0.15).

1.8 HorRat Value

The ratio of the reproducibility relative standard deviation calculated from the data to the PRSD(R) calculated from the Horwitz formula:

$$HorRat = RSD(R)/PRSD(R)$$

To differentiate the usual HorRat value calculated from reproducibility data from the HorRat value calculated from repeatability data, attach an R for the former and an r for the latter. But note that the denominator always uses the PRSD(R) calculated from reproducibility data because this parameter is more predictable than the parameter calculated from repeatability data:

$$HorRat(R) = RSD_R/PRSD(R)$$

$$HorRat(r) = RSD_r/PRSD(R)$$

Some expected, predicted relative standard deviations are given in Table D1.

2 *Acceptable HorRat Values*

2.1 For Interlaboratory Studies

HorRat(R): The original data developed from interlaboratory (among-laboratory) studies assigned a HorRat value of 1.0 with limits of acceptability of 0.5 to 2.0. The corresponding within-laboratory relative standard deviations were found to be typically 1/2 to 2/3 the among-laboratory relative standard deviations.

Table D2. Predicted relative standard deviations

Concentration (C)	$PRSD_R$, %	$PRSD_r$, %
100%	2	1
1%	4	2
0.01%	8	4
1 ppm	16	8
10 ppb	32	16
1 ppb	45	22

2.1.1 Limitations

HorRat values do not apply to method-defined (empirical) analytes (moisture, ash, fiber, carbohydrates by difference, etc.), physical properties or physical methods (pH, viscosity, drained weight, etc.), and ill-defined analytes (polymers, products of enzyme reactions).

2.2 For Intralaboratory Studies

2.2.1 Repeatability

Within-laboratory acceptable predicted target values for repeatability are given in Table D2 at 1/2 of PRSD(R), which represents the best case.

2.2.2 HorRat(r)

Based on experience and for the purpose of exploring the extrapolation of HorRat values to SLV studies, take as the minimum acceptability 1/2 of the lower limit ($0.5 \times 0.5 \approx 0.3$) and as the maximum acceptability 2/3 of the upper limit ($0.67 \times 2.0 \approx 1.3$).

Calculate HorRat(r) from the SLV data:

$$HorRat(r) = RSD(r)/PRSD(R)$$

Acceptable HorRat(r) values are 0.3–1.3. Values at the extremes must be interpreted with caution. With a series of low values, check for unreported averaging or prior knowledge of the analyte content; with a series of high values, check for method deficiencies such as unrestricted times, temperatures, masses, volumes, and concentrations; unrecognized impurities (detergent residues on glassware, peroxides in ether); incomplete extractions and transfers and uncontrolled parameters in specific instrumental techniques.

2.3 Other Limitations and Extrapolations

The HorRat value is a very rough but useful summary of the precision in analytical chemistry. It overestimates the precision at the extremes, predicting more variability than observed at the high end of the scale (C > ca 0.1; i.e., >10%) and at the low end of the scale (C < E-8; i.e., 10 ng/g; 10 ppb).

ANNEX E
AOAC Method Accuracy Review

Accuracy of Method Based on Reference Material

Reference material (RM) used.—The use of RMs should be seen as integral to the process of method development, validation, and performance evaluation. RMs are not the only component of a quality system, but correct use of RMs is essential to appropriate quality management. RMs with or without assigned quantity values can be used for measurement precision control, whereas only RMs with assigned quantity values can be used for calibration or measurement trueness control. Method development and validation for matrices within the scope of the method is done to characterize attributes such as recovery, selectivity, "trueness" (accuracy, bias), precision (repeatability and reproducibility), uncertainty estimation, ruggedness, LOQ or LOD, and dynamic range. RMs should be chosen that are fit-for-purpose. When certified reference materials (CRMs) are available with matrices that match the method scope, much of the work involved in method development has already been completed, and that work is documented through the certificate. RMs with analyte values in the range of test samples, as well as "blank" matrix RMs, with values below or near detection limits, are needed.

Availability of RM.—Consideration needs to be given to the future availability of the chosen RM. Well-documented methods that cannot be verified in the future due to lack of material may lose credibility or be seen as inferior.

Fit to method scope.—Natural matrix CRMs provide the greatest assurance that the method is capable of producing accurate results for that matrix. When selecting an RM to perform a method validation, analysts should consider the method to material fit. An example of a good fit would be a method for specified organic molecules in infant formula and using an infant formula or powder milk RM. A poor fit would be a method for specified organic molecules in infant formula and using a sediment material.

Stability.—Providing a stable RM can be challenging where analytes are biologically active, easily oxidized, or interactive with other components of the matrix. CRM producers provide assurance of material stability, as well as homogeneity.CRMs are accompanied by a certificate that includes the following key criteria:

(*1*) Assigned values with measurement uncertainty and metrological traceability
(*2*) Homogeneity
(*3*) Stability, with the expiration date for the certificate
(*4*) Storage requirements
(*5*) Information on intended use
(*6*) Identity of matrix

For some RMs, such as botanical RMs, the source and/or authenticity can be a very important piece of information that should be included with the certificate. Even under ideal storage conditions, many analytes have some rate of change. Recertification may be done by the supplier, and a certificate reissued with a different expiration date and with certain analyte data updated or removed.

Definition of CRM.—Refer to the AOAC TDRM document for definitions from ISO Guide 30, Amd. 1 (2008), http://www.aoac.org/divisions/References.pdf.

Information on source of RM is available.—It is the responsibility of the material producer to provide reliable authentication of the RM and make a clear statement in the accompanying documentation. This should be an as detailed listing as possible, including handling of ingredients, identification of plant materials as completely as feasible (species, type, subtype, growing region), etc. This is comparable to other required information on an RM for judging its suitability for a specific application purpose (e.g., containing how much of the targeted analyte, stabilized by adding acid—therefore not suited for certain parameters/procedures, etc.).

Separate RM used for calibration and validation.—A single RM cannot be used for both calibration and validation of results in the same measurement procedure.

Blank RM used where appropriate.—Blank matrix RMs are useful for ensuring performance at or near the detection limits. These are particularly useful for routine quality control in methods measuring, for instance, trace levels of allergens, mycotoxins, or drug residues.

Storage requirements were maintained.—Method developers should maintain good documentation showing that the RM producer's recommended storage conditions were followed.

Cost.—The cost of ongoing method checks should be considered. Daily use of CRMs can be cost prohibitive. Monthly or quarterly analysis of these materials may be an option.

Concentration of analyte fits intended method.—Concentration of the analyte of interest is appropriate for standard method performance requirements (SMPRs).

Uncertainty available.—Every measurement result has an uncertainty associated with it, and the individual contributions toward the combined uncertainty arise from multiple sources. Achieving the target measurement uncertainty set by the customer for his/her problem of interest is often one of the criteria used in selecting a method for a given application. Estimation of measurement uncertainty can be accomplished by different approaches, but the use of RMs greatly facilitates this part of a method validation.

Demonstration of Method Accuracy when No Reference Material Is Available

If an RM is not available, how is accuracy demonstrated?

There are many analytes for which a CRM with a suitable matrix is not available. This leaves the analyst with few options. For some methods, there may be proficiency testing programs that include a matrix of interest for the analyte. Proficiency testing allows an analyst to compare results with results from other laboratories, which may or may not be using similar methods. Spiking is another technique that may be used. When alternative methods are available, results may be compared between the different methods. These alternatives do not provide the same level of assurance that is gained through the use of a CRM.

Spike recovery.—In the absence of an available CRM, one technique that is sometimes used for assessing performance is the spiking of a matrix RM with a known quantity of the analyte. When this method is used, it cannot be assumed that the analyte is bound in the same way as it would be in a natural matrix. Nevertheless, a certified blank RM would be the preferred choice for constructing a spiked material.

When preparing reference solutions, the pure standards must be completely soluble in the solvent. For insoluble materials in a liquid suspension or for powdered forms of dry materials, validation is required to demonstrate that the analyte is homogeneously distributed and that the response of the detection system to the analyte is not affected by the matrix or preparation technique. When a matrix material is selected for spiking, it should be reasonably

The document, *AOAC Method Accuracy Review*, was prepared by the AOAC Technical Division on Reference Materials (TDRM) and approved by the AOAC Official Methods Board in June 2012.

characterized to determine that it is sufficiently representative of the matrix of interest. Spiked samples must be carried through all steps of the method. Many analytes are bound in a natural matrix and whether the spiked analyte will behave the same as the analyte in a natural matrix is unknown.

Other.—Use of a substitute RM involves the replacement of the CRM with an alternative matrix RM matching the matrix of interest as close as possible based on technical knowledge.

ANNEX F
Development and Use of In-House Reference Materials

The use of reference materials is a vital part of any analytical quality assurance program. However, you may have questions about their creation and use. The purpose of this document is to help answer many of these questions.

- What is a reference material?
- Why use reference materials?
- What certified reference materials are currently available?
- Why use an in-house reference material?
- How do I create an in-house reference material?
- How do I use the data from an in-house reference material?

What Is a Reference Material?

The International Organization for Standardization (ISO) defines a reference material as a "material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials" (1). In plain English, natural-matrix reference materials, such as those you might prepare for use in-house, can be used to validate an analytical method or for quality assurance while you're using your method to analyze your samples. (Natural-matrix materials are not generally used as calibrants because of the increased uncertainty that this would add to an analysis.) The assigned values for the target analytes of an in-house reference material can be used to establish the precision of your analytical method and, if used in conjunction with a CRM, to establish the accuracy of your method.

ISO defines a certified reference material (CRM) as a "reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence" (1).

Why Use Reference Materials?

Certified reference materials can be used across the entire scope of an analytical method and can provide traceability of results to the International System of Units (SI). During method development, CRMs can be used to optimize your method. During method validation, they can be used to ensure that your method is capable of producing the "right" answer, and to determine how close your result is to that answer. During routine use, they can be used to determine within-day and between-day repeatability, and so demonstrate that your method is in control and is producing accurate results every time it is used.

Natural-matrix reference materials should mimic the real samples that will be analyzed with a method. They should behave just as your samples would during a procedure, so if you obtain accurate and precise values for your reference material, you should obtain accurate and precise values for your samples as well.

What Certified Reference Materials Are Currently Available?

CRMs are available from a number of sources, including (but not limited to):

- American Association of Cereal Chemists (AACC)
- American Oil Chemists Society (AOCS)
- International Atomic Energy Agency (IAEA)
- Institute for Reference Materials and Measurements (IRMM)
- LGC Promochem
- National Institute of Standards and Technology (NIST)
- National Research Council Canada (NRC Canada)
- UK Food Analysis Proficiency Assessment Program (FAPAS)

A number of websites provide general overviews and catalogs of producers' and distributors' reference materials:

http://www.aocs.org/tech/crm/
http://www.comar.bam.de
http://www.erm-crm.org
http://www.iaea.org/oregrammeslaqcs
http://www.aaccnet.org/checksample
http://www.irmm·ire.be/mrm.html
http://www.lgcpromochem.com
http://www.naweb.iaea.org/nahu/nmrm/
http://www.nist.gov/srm
http://www.fapas.com/index. cfm
http://www.virm.net.

Because new reference materials are produced regularly, it is important to check these websites to determine what is currently available.

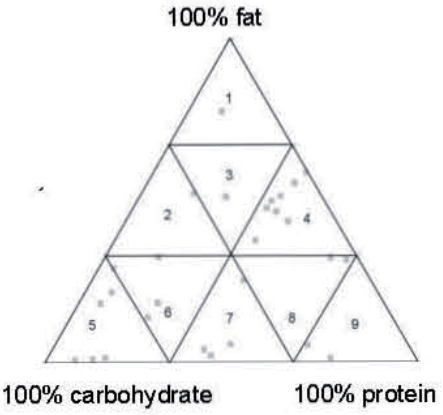
Why Use an In-House Reference Material?

There are many benefits to the use of a CRM. CRMs have been prepared to be homogeneous and, if stored under the proper conditions, stable. You are provided with a certified value as well as the statistical data for theconcentration of your analyte; this is about as close as you can come to knowing the true value of the concentration of the analyte. The material has been tested by experienced analysts in leading laboratories, so you have the security of knowing that your method is generating values similar to those generated in other competent laboratories. The CRMs from the sources mentioned above are nationally and/or internationally recognized, so when you obtain acceptable results for a CRM using your analytical method, you give credibility to your methodology and traceability to your results.

But there are some drawbacks associated with CRMs. Unfortunately, many analyte/matrix combinations are not currently available. When testing food products for nutrient content, for example, a laboratory can be asked to analyze anything that might be found in a kitchen or grocery store. Reference materials that represent all of the types of foods that need to be tested are not available, and most CRMs are certified for a limited number of analytes. It is important to match the reference material matrix to your sample matrix. (Food examples dominate the discussion below, but the same processes apply to the development of in-house RMs in other areas of analytical chemistry.)

To demonstrate the applicability of an analytical method to a wide variety of food matrices, AOAC INTERNATIONAL's Task

Excerpted from *Development and Use of In-House Reference Materials*, Rev. 2, 2009. Copyright 2005 by the AOAC Technical Division on Reference Materials (TDRM).



Force on Methods for Nutrition Labeling developed a triangle partitioned into sectors in which foods are placed based on their protein, fat, and carbohydrate content (2, 3). Since ash does not have a great impact on the performance of an analytical method for organic-material foods, and water can be added or removed, it can be assumed that the behavior of an analytical method is determined to large extent by the relative proportions of these proximates. AOAC INTERNATIONAL anticipated that one or two foods in a given sector would be representative of other foods in that sector and therefore would be useful for method assessment. Similarly, one or two reference materials in a given sector (or near each other in adjacent sectors) should be useful for quality assurance for analyses involving the other foods in the sector. The positions of many of the food-matrix CRMs from the sources listed above are shown in the triangle and are provided in the list.

These food-matrix reference materials are spread through all sectors of the triangle, thereby making it likely that you can find an appropriate CRM to match to your samples. Ultimately, however, the routine use of a CRM can be cost prohibitive, and is not really the purpose of CRMs. For example, in order to use NIST's Standard Reference Material (SRM) 2387 Peanut Butter for all mandatory nutrition labeling analyses, you could buy one sales unit (three jars, each containing 170 g material) for $649 (2009 price). If you charge your customer about $1000 for analysis of all mandatory nutrients in a test material, the control material would account for more than 60% of your fees. Therefore, many laboratories have found it more cost-effective to create in-house reference materials for routine quality control and characterize them in conjunction with the analysis of a CRM (4). You can prepare larger quantities of a reference material by preparing it in-house, and you have more flexibility in the types of matrices you can use. There are not many limitations on what can be purchased.

How Do I Create an In-House Reference Material?

There are basically three steps to preparing an in-house reference material: selection (including consideration of homogeneity and stability), preparation, and characterization. Additional guidance through these steps can be provided from TDRM as well as in ISO Guides 34 (5) and 35 (6).

References

(1) JCGM 200:2008, *International vocabulary of metrology—Basic and general concepts and associated terms (VIM)*, International Bureau of Weights and Measures (www.bipm.org)

Sector	RM No.	Matrix
	NIST 1563	Coconut oil
1	NIST 3274	Fatty acids in botanical oils
1	NIST 3276	Carrot extract in oil
1	LGC 7104	Sterilized cream
2	NIST 2384	Baking chocolate
3	NIST 2387	Peanut butter
4	NIST 1546	Meat homogenate
4	LGC 7106	Processed cheese
4	LGC 7000	Beef/pork meat
4	LGC 7150	Processed meat
4	LGC 7151	Processed meat
4	LGC 7152	Processed meat
4	SMRD 2000	Fresh meat
4	LGC 7101	Mackerel paste
4	LGC QC1001	Meat paste 1
4	LGC QC1004	Fish paste 1
5	BCR-382	Wleat flour
5	BCR-381	Rye flour
5	LGC 7103	Sweet digestive biscuit
5	LGC 7107	Madeira cake
5	LGC QC1002	Flour 1
6	NIST 1544	Fatty acids
6	NIST 1548a	Typical diet
6	NIST 1849	Infant/adult nutritional formula
6	LGC 7105	Rice pudding
7	LGC 7001	Pork meat
7	NIST 1566b	Oyster tissue
7	NIST 1570a	Spinach leaves
7	NIST 2385	Spinach
8	NIST 1946	Lake trout
8	LGC 7176	Canned pet food
9	NIST 1974a	Mussel tissue
9	NIST 3244	Protein powder

(2) Wolf, W.R., & Andrews, K.W. (1995) *Fresenius' J. Anal. Chem.* **352**, 73–76
(3) Wolf, W.R. (1993) *Methods of Analysis for Nutrition Labeling*, D.R. Sullivan & D.E. Carpenter (Eds), AOAC INTERNATIONAL, Gaithersburg, MD
(4) European Reference Materials (2005) *Comparison of a Measurement Result with the Certified Value*, Application Note 1
(5) *ISO Guide 34 General Requirements for the Competence of Reference Material Producers* (2009) 2nd, International Organization for Standardization, Geneva, Switzerland
(6) *Guide 35 Certification of Reference Materials—General and Statistical Principles* (2006) International Organization for Standardization, Geneva, Switzerland

For more information about the AOAC Technical Division on Reference Materials, visit http://aoac.org/divisions/tdrm.

Appendix G: Procedures and Guidelines for the Use of AOAC Voluntary Consensus Standards to Evaluate Characteristics of a Method of Analysis

Expert Review Panels, Official Methods Board, First and Final Action *Official Methods*℠

In early 2011, an AOAC Presidential Task Force recommended that AOAC use Expert review panels (ERPs) to assess candidate methods against standard method performance requirements (SMPRs) to ensure that adopted First Action Official Methods℠ are fit for purpose.

Formation of an ERP

AOAC ERPs are authorized to adopt candidate methods as First Action *Official Methods* and to recommend adoption of these methods to Final Action *Official Methods* status. Scientists are recruited to serve on ERPs by a variety of ways. Normally, a call for experts is published at the same time as a call for methods is posted. Interested scientists are invited to submit their *curriculum vitae* (CV) for consideration. Advisory panel, stakeholder panel, and working group members may make recommendations to AOAC for ERP members. All CVs are reviewed and evaluated for expertise by the AOAC Chief Scientific Officer (CSO). The CVs and CSO evaluations are forwarded to the OMB for formal review. Both the CSO and OMB strive to ensure that the composition of a proposed ERP is both qualified and represent the various stakeholder groups. The recommended ERP members are submitted to the AOAC president who then appoints the ERP members.

Review of Methods

Methods submitted to AOAC in response to a call for methods are collected and compiled by AOAC staff. The AOAC CSO and working group chair perform a preliminary review of the methods and classify them into three categories: (*1*) fully developed and written methods that appear to meet SMPRs; (*2*) fully developed and written methods that may or may not meet SMPRs; and (*3*) incomplete methods with no performance data. Method submitters are apprised of the evaluation of their methods. Method developers with submissions that are classified as Category 2 or 3 are encouraged to provide additional information if available. A list of all the submitted methods and their classifications are posted for public review.

Usually, two ERP members (sometimes more) are assigned to lead the review of each Category 1 method. An ERP meeting is convened to review the methods. ERP meetings are open to all interested parties, and are usually well-attended events with about 50–60 attendees common. Each Category 1 method is reviewed and discussed by the ERP. If stakeholders have designated the method to be a dispute resolution method (as stated in the SMPR), then the ERP is asked to identify the single best candidate method to be adopted as a First Action *Official Method*. If the SMPR does not specify the need for a dispute resolution method, then the ERP may choose to adopt all methods that meet the SMPRs, or may choose to adopt the single best method in their collective, expert opinion.

In addition, an ERP may choose to require changes to a candidate method as part of its First Action adoption and/or identify issues that are required to be resolved prior to adoption as a Final Action *Official Method.*

Methods adopted by an ERP as First Action *Official Methods* may not be in AOAC *Official Methods* format. Method developers/authors are asked to assist AOAC to rewrite the method and accompanying manuscript into an AOAC-acceptable format.

Two-Year First Action Evaluation Period

Under the new pathway, a method may be designated as a First Action *Official Method* based on the collective judgment of an ERP. *Official Methods* remain as First Action for a period of about 2 years. During the First Action period, the method will be used in laboratories, and method users will be asked to provide feedback on the performance of the method.

As previously described, two (or more) ERP members are assigned to lead the review of candidate methods for adoption as First Action *Official Methods*. After a method has been adopted as First Action, these lead reviewers are expected to keep track of the use of and experience with the First Action *Official Method*. At the conclusion of the 2-year evaluation period, one or both of the lead reviewers will report back to the ERP on the experience of the First Action *Official Method.*

The presiding ERP will monitor the performance of the method, and, at the completion of the 2-year First Action evaluation period, determine whether the method should be recommended to the OMB for adoption as an AOAC Final Action *Official Method*.

It is also possible that First Action *Official Methods* are not recommended for Final Action. There are two possibilities for an ERP to decide not to proceed with a First Action method: (*1*) feedback from method users indicates that a First Action method is not performing as well in the field as was expected; or (*2*) another method with better performance characteristics has been developed and reviewed. In either case, the ERP may choose to repeal the First Action status of a method.

OMB Review

The OMB will review all methods recommended for Final Action or repeal by the ERP, and will consider a number of factors in their decision. A guidance document for factors to consider is provided on the AOAC website at http://www.aoac.org/vmeth/OMB_ERP_Guidance.pdf. Some of the factors identified by the guidance document for OMB consideration are (*1*) feedback from method users, (*2*) comparison to the appropriate SMPR, (*3*) results from single-laboratory validation, (*4*) reproducibility/uncertainty and probability of detection, (*5*) availability of reference materials, and (*6*) safety concerns.

Conclusion

The new pathway to *Official Methods*℠ is deliberately designed to avoid creation of elaborate review systems. The intent of the model is for method experts to use their scientific knowledge, experience, and good judgment to identify and adopt the best methods possible for the analytical need.

These methods are then published as First Action *Official Methods*, and used by analysts while additional information about the method is collected.

Method reviewers may consider other forms of information in lieu of the traditional collaborative study to demonstrate method reproducibility.

Additional Information

Coates, S. (2012) "Alternative Pathway," *Inside Laboratory Management* **16**(3), pp 10–12

Expert Review Panels, Policies and Procedures, AOAC INTERNATIONAL, http://www.aoac.org/News/EXPERT%20REVIEW%20PANELS%20final%20revision.pdf

Standard Format and Guidance for AOAC Standard Method Performance Requirement (SMPR) Documents, AOAC INTERNATIONAL, http://www.aoac.org/ISPAM/pdf/3.5%20SMPR%20Guideline%20v12.1.pdf

Guidance Documents

Requirements for First Action Official Methods℠ Status

See Figure 1 for process flowchart.

Expert Review Panels

(*1*) Supported by relevant stakeholders.

(*2*) Constituted solely for the ERP purpose, not for SMPR purposes or as an extension of an SMPR.

(*3*) Consist of a minimum of seven members representing a balance of key stakeholders.

(*4*) ERP constituency must be approved by the OMB.

(*5*) Hold transparent public meetings only.

(*6*) Remain in force as long as method in First Action status.

First Action Official Method℠ Status Decision

(*1*) Must be made by an ERP constituted or reinstated post March 28, 2011 for First Action *Official Method℠* status approval.

(*2*) Must be made by an ERP vetted for First Action *Official Method℠* status purposes by OMB post March 28, 2011.

(*3*) Method adopted by ERP must perform adequately against the SMPR set forth by the stakeholders.

(*4*) Method must be adopted by unanimous decision of ERP on first ballot. If not unanimous, negative votes must delineate scientific reasons.

(*5*) Negative voter(s) can be overridden by 2/3 of voting ERP members after due consideration.

(*6*) Method becomes Official First Action on date when ERP decision is made.

(*7*) Methods to be drafted into AOAC format by a knowledgeable AOAC staff member or designee in collaboration with the ERP and method author.

(*8*) Report of First Action *Official Method℠* status decision complete with ERP report regarding decision, including scientific background (references, etc.), to be published concurrently with method in traditional AOAC publication venues.

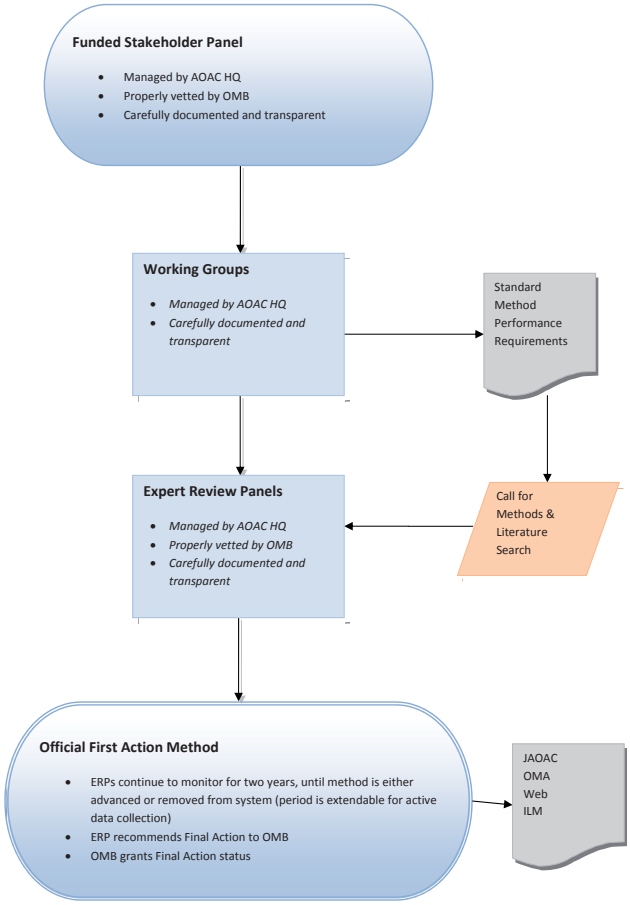


Figure 1. Summary of standards development through *Official Methods of Analysis*.

Method in First Action Status and Transitioning to Final Action Status

(*1*) Further data indicative of adequate method reproducibility (between laboratory) performance to be collected. Data may be collected via a collaborative study or by proficiency or other testing data of similar magnitude.

(*2*) Two years maximum transition time [additional year(s) if ERP determines a relevant collaborative study or proficiency or other data collection is in progress].

(*3*) Method removed from Official First Action and OMA if no evidence of method use available at the end of the transition time.

(*4*) Method removed from Official First Action and OMA if no data indicative of adequate method reproducibility is forthcoming as outlined above at the end of the transition time.

(*5*) ERP to recommend method to Final Action Official status to the OMB.

(*6*) OMB decision on First to Final Action status.

These guidance documents were approved by the AOAC Board of Directors on May 25, 2011.

First Action to Final Action Methods: Guidance for AOAC Expert Review Panels

In December 2011, the Official Methods Board (OMB) approved a guidance document for ERPs to support their work as they deliberate on methods, adopt methods as Official First Action, and, subsequently, track method usage and performance between First Action status and Final Action consideration. The guideline is based on parameters of a method that the OMB will consider when deliberating on methods recommended for Final Action status. ERPs are to use this guideline in their deliberations.

ERPs working within the AOAC process may recommend a First Action status method be elevated to Final Action status. Such a recommendation leverages the ERP's high level of expertise supported by data from the initial evaluation, and results from the subsequent 2-year method performance evaluation period.

The OMB receives the recommendation with supporting documentation, and determines if Final Action status is warranted. OMB's review verifies the method process was conducted in compliance with the guidelines and protocols of the Association.

For transparency and to expedite the review process, the main areas OMB will review when evaluating ERP recommendations to promote methods to Final Action are listed below. Documentation of the areas listed below will also increase confidence in method performance and assist users to properly and safely perform the methods at their locations.

A. Method Applicability

(**a**) A method's applicability to the identified stakeholder needs is best assessed by the stakeholder panel and should be a part of the process from the onset. OMB liaisons will remind stakeholder panels to maintain this focus point.

(**b**) OMB may ask ERPs and stakeholder panels for feedback to improve the applicability of the method, such as potential method scope expansions and potential points of concern.

B. Safety Concerns

(**a**) A safety review must be performed for a method to be recognized as First Action.

(**b**) All safety concerns identified during the 2-year evaluation period must be addressed.

(**c**) Guidance and support can be obtained from the AOAC Safety Committee.

C. Reference Materials

(**a**) Document efforts undertaken to locate reference materials. Methods may still progress to Final Action even if reference materials are not available.

(**b**) Guidance and support can be obtained from the AOAC Technical Division on Reference Materials.

D. Single-Laboratory Validation

(**a**) Data demonstrating response linearity, accuracy, repeatability, LOD/LOQ, and matrix scope must be present. Experimental designs to collect this data may vary with the method protocol and the intended use of the method.

(**b**) Resources can be identified by the AOAC Statistics Committee.

E. Reproducibility/Uncertainty and Probability of Detection

(**a**) For quantitative methods, data demonstrating reproducibility and uncertainty must be present. Experimental designs to collect this data may vary with the method protocol, available laboratories, and the intended use of the method (i.e., collaborative studies, proficiency testing, etc.).

(**b**) For qualitative methods, data must be present demonstrating the probability of detection at specified concentration levels as defined by the SMPR. Experimental designs to collect this data may vary with the method protocol, available laboratories, and the intended use of the method.

(**c**) Guidance and support can be obtained from the AOAC Statistics Committee.

F. Comparison to SMPR

(**a**) Document method performance versus SMPR criteria. Note which SMPR criteria are met. For SMPR criteria not met, the ERP documents the reasoning why the method is still acceptable.

(**b**) Data is present to assure the matrix and analyte scopes are covered. This is critical for methods used for dispute resolutions.

G. Feedback from Users of Method

(**a**) Document positive and negative feedback from users of the method during the trial period.

(**b**) Feedback from users demonstrating method ruggedness should be documented.

(**c**) Assess the future availability of vital equipment, reference materials, and supplies.

H. ERP Recommendations to Repeal First Action Methods

Recommendations to repeal First Action methods shall be accompanied with detailed reasons for the decision.

The First to Final Action guidance for ERPs was approved by the OMB in December 2011 and effective as of February 1, 2012.