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STAKEHOLDER PANEL ON DIETARY SUPPLEMENTS –
EXPERT REVIEW PANELS

ALOIN | CINNAMON | TEA

The Scientific Association Dedicated to Analytical Excellence®

TUESDAY, AUGUST 2, 2016

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The Scientific Association Dedicated to Analytical Excellence®

AOAC Stakeholder Panel on Dietary Supplements

EXPERT REVIEW PANEL – ALOIN, CINNAMON, AND TEA

Tuesday, August 2, 2016

A G E N D A

EXPERT REVIEW PANEL CHAIR: Brian Schaneberg, Starbucks

1. Welcome and Introductions (9:00 a.m. – 9:10 a.m.)
   Brian Schaneberg, Starbucks (ERP Chair)

2. Review
   a. AOAC Volunteer Policies & ERP Proccess Overview and Guidelines (9 :10 a.m. – 9:30 a.m.)
      Deborah McKenzie

3. Review of Methods

   For each method the assigned ERP members will present a review of the revised method manuscripts, after which the ERP will discuss the method and render a decision on the status for each method.

   A. Aloin
      a. ALN-01
      b. ALN-02
      c. Final Action Requirements for Approved Method(s)

   B. Cinnamon
      a. CIN-01
      b. Final Action Requiremets for Approved Method(s)

   C. Tea
      a. TEA-01
      b. TEA-02
      c. Final Action Requiremets for Approved Method(s)

1. Adjourn (3 :00 p.m.)

SPDS Set 3 ERP - Aloin				
Chair	Brian Schaneberg	Starbucks Coffee Company	USA	bschaneb@starbucks.com
Member	Nour Eddine Es-Safi	Mohammed V University in Rabat	Morocco	nouressafi@yahoo.fr
Member	Quanyin Gao	Herbalife International Of America	USA	quanying@herbalife.com
Member	Holly Johnson	Alkemist Labs	USA	holly@alkemist.com
Member	Philip Koerner	Phenomenex	USA	PhilK@Phenomenex.com
Member	Jasen Lavoie	Aloe Council	USA	JLavoie@Pharmachemlabs.com
Member	Charles Metcalfe	Custom Analytics	USA	cem@calabs.us
Member	Tom Phillips	MD Department Of Agriculture	USA	tom.phillips@maryland.gov
Member	Klaus Reif	PhytoLab GmbH & Co., KG	Germany	klaus.reif@phytolab.de
Member	Aniko Solyom	GAAS Analytical	USA	asolyom@gaasanalytical.com
Member	Darryl Sullivan	Covance Laboratories	USA	darryl.sullivan@covance.com
Member	Jianming Tao	Spectrix Analytical Services, LLC	USA	jtao@spectrixservices.com
Member	Marina Torres	Laboratorio Tecnologico Del Urugu	Uruguay	mtorres@latu.org.uy

AOAC SPDS Set 3 ERP - Cinnamon				
Chair	Brian Schaneberg	Starbucks Coffee Company	USA	bschaneb@starbucks.com
Member	Milda Embuscado	McCormick	USA	Milda_Embuscado@mccormick.com
Member	Nour Eddine Es-Safi	Mohammed V University in Rabat	Morocco	nouressafi@yahoo.fr
Member	John Finley	Louisiana State University	USA	jfinley@agcenter.lsu.edu
Member	Martha Jennens	Covance Laboratories	USA	martha.jennens@covance.com
Member	Tom Phillips	MD Department Of Agriculture	USA	tom.phillips@maryland.gov
Member	Darryl Sullivan	Covance Laboratories	USA	darryl.sullivan@covance.com
Member	Joseph Zhou	Sunshineville Health Products, Inc	USA	josephzhou@sunshinevillehp.com

SPDS Set 3 ERP - Tea				
Chair	Brian Schaneberg	Starbucks Coffee Compan	USA	bschaneb@starbucks.com
Member	Nour Eddine Es-Safi	Mohammed V University i	Morocco	nouressafi@yahoo.fr
Member	Tetsuhisa Goto	Shinshu University	JAPAN	tetsuhisagoto@yahoo.co.jp
Member	Martha Jennens	Covance Laboratories	USA	martha.jennens@covance.com
Member	Holly Johnson	Alkemist Labs	USA	holly@alkemist.com
Member	Philip Koerner	Phenomenex	USA	PhilK@Phenomenex.com
Member	Melissa Phillips	NIST	USA	melissa.phillips@nist.gov
Member	Tom Phillips	MD Department Of Agricu	USA	tom.phillips@maryland.gov
Member	Klaus Reif	PhytoLab GmbH & Co., KG	Germany	klaus.reif@phytolab.de
Member	Aniko Solyom	GAAS Analytical	USA	asolyom@gaasanalytical.com
Member	Darryl Sullivan	Covance Laboratories	USA	darryl.sullivan@covance.com
Member	Jinchuan Yang	Waters Corporation	USA	jinchuan_yang@waters.com
Member	Kurt Young	GNC/Nutra Manufacturin	USA	kurt.young@nutramfg.com

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AOAC Stakeholder Panel on Dietary Supplements Expert Review Panel

AOAC Candidate Method #ALN-01

Determination of Aloin A and Aloin B in Aloe vera Raw Materials and Finished Products by High-Performance Liquid Chromatography: Single-Laboratory Validation

- Author(s): Paula N. Brown, Ronan Yu, Chiow Hui Kuan, Jamie Finley, Elizabeth M. Mudge, Seven Dentali
- Submitted by: Elizabeth Mudge, BCIT
- Enclosures: 1
- Submitter notes: None

Link to Method: http://griegler-aoac-org.cld.bz/ALN-01
Password: aoac1

Primary Reviewer: Jason Lavoie

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-28 13:46:23
Name	Nour Eddine ES-SAFI
E-mail	nouressafi@yahoo.fr
Organization	Mohammed V University in Rabat
Title of Method	Determination of Aloin A and Aloin B in Aloe vera Raw Materials and Finished Products by High-Performance Liquid Chromatography: Single-Laboratory Validation
AOAC Candidate Method Number (e.g. ALN-01)	ALN-1
Applicable SMPR	AOAC SMPR 2015.015
Summary:	The proposed methods presents results dealing with the quantitative analysis of aloin A and aloin B in Aloe vera raw material and finished products. The method used HPLC technique and separation was acheived on a C18 column using isocratic elution. Aloin A and aloin B were detected at 357 nm using a DAD detector.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes. In agreement to what is specified in the applicability of the SMPR the method concerns the quantitative analysis of aloin A and B in several matrixes such as Aloe vera dry juices, Aloe vera leaves juices, ...
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	* Analytical range: 0.3 µg/mL to 50 µg/mL. * LOQ: 0.23 µg/mL (Aloin A) and 0.21 µg/mL (Aloin B). Values indicated in the SMR tables are 0.005 ppm. * LOD: 0.09 µg/mL (Aloin A and B) * Repeatability: RSD values ranging from 1.09 to 8.64 % which seems in agreement with the SMPR table values. * Recovery: the average recovery range of aloin A and aloin B raging from 84.41 to 108.86 %. I think that some of the obtained values are out of those indicated in the SMPR. * Reproducibility: not conducted.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes. In general the definitions specified in the SMPR were appropriately used in the proposed method.
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	No, no the Reference Materials stated in the SMPR have been used in the proposed method.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	No, no the Reference Materials stated in the SMPR have been used in the proposed method.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	The analytes reported in the SMPR are aloin A and B. LOD, LOQ, repeatability and recovery analysis have been performed on both analytes aloin A and aloin B. Analytical range and reproducibility are missing.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Yes, the proposed method uses injection of reference standards;
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	Yes
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes
5. Based on the supporting information, what are the pros/strengths of the method?	The method is simple robust allowing a good separation of the two investigated aloins in a relatively short time.
6. Based on the supporting information, what are the cons/weaknesses of the method?	The purity of the separated compounds should be checked.
7. Any general comments about the method?	No
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	I don't think that this method could be adopted as a first action in the present form. I think that it could be enhanced by more experimental data.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-30 17:44:33
Name	Phil Koerner
E-mail	philk@phenomenex.com
Organization	Phenomenex
Title of Method	Determination of Aloin A and Aloin B in Aloe vera Raw Materials and Finished Products by High-Performance Liquid Chromatography: Single-Laboratory Validation
AOAC Candidate Method Number (e.g. ALN-01)	ALN-01
Applicable SMPR	2015.015
Summary:	HPLC-UV isocratic method for determination of Aloin A and Aloin B in raw materials and finished products
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	yes
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	safety related to reagents used: "consult MSDS"; although reagents are typical for HPLC method and expect typical lab to be aware of precautions to take
1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	yes

2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	yes
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	reported LOD (0.09 ppm) and LOQ (0.2 ppm) not as low as SMPR stated requirement of 0.005 ppm; expect this could be improved?
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	yes
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	no
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	QC sample defined; should use as system suitability standard for a) chromatographic resolution b/w Aloin A and B (>/= 2.0), b) peak symmetry, and c) %RSD

3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	yes for resolution
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	yes
5. Based on the supporting information, what are the pros/strengths of the method?	fast, straight forward HPLC method, no special equipment required
6. Based on the supporting information, what are the cons/weaknesses of the method?	1) core-shell 2.6um column used on HPLC system; was optimization of HPLC system (faster data acquisition and decrease in extra column volume (e.g. connecting tubing) performed?
7. Any general comments about the method?	method appears to be well suited to meet most requirements outline in SMPR 2015.015
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	yes; meets most requirements outline in SMPR 2015.015 - if LOD and LOQ requirements could be met with minor adjustment to method

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-31 21:35:32
Name	charles metcalfe
E-mail	laptop@calabs.us
Organization	custom analytics
Title of Method	Determination of Aloin in Aloe Vera
AOAC Candidate Method Number (e.g. ALN-01)	ALN-01
Applicable SMPR	2015.015
Summary:	Reverse phase HPLC with UV detection at 355 nm
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	The method is fine except for the LOD is only 0.1 ppm and LOQ is 0.3 ppm.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	The SPMR requires much lower levels but with minor method changes (ie injection volume) the levels could be greatly reduced.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	No, but the reagents and instrumentation used are non-hazardous.
1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes

2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	yes
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	yes
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	yes
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	No system suitability is mentioned

3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	No system suitability is mentioned
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes. A very simple method with no complicated sample prep.
5. Based on the supporting information, what are the pros/strengths of the method?	Very simple and easy to perform.
6. Based on the supporting information, what are the cons/weaknesses of the method?	Detection / quantitaion limits are too high. Easily corrected by increasing injection volume.
7. Any general comments about the method?	Great method, just needs more data for lower limits.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	If more data is submitted to support lower limits of detection / quantitation. Minimal additional work required.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-13 10:34:20
Name	Klaus Reif
E-mail	klaus.reif@phytolab.de
Organization	PhytoLab GmbH & CO. KG
Title of Method	Determination of Aloin A and Aloin B in Aloe vera Raw Materials and Finished Products by HPLC
AOAC Candidate Method Number (e.g. ALN-01)	ALN-01
Applicable SMPR	SMPR 2015.015
Summary:	The method is for the Determination of both aloin A and aloin B by HPLC-UV, applicable to raw materials and finished products.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	YES
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	YES
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	YES
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	NO, "Review Safety Data Sheests for all reagents and chemicals. Follow manufacturers' maulans ans instructions while running HPLC and other devices" (taken from TEA-01)
1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	YES

2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	There are no specicic Reference Materials like NIST-samples etc. described in the SMPRs
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	s. question 2
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	YES
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	NO, yes I would recommend a blank injection and an repeated control injections with Standards at the LOQ and in a middle range. (tbd)

3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	s. question 2
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	YES
5. Based on the supporting information, what are the pros/strengths of the method?	Commercial and common Equipment, conventional HPLC used, modern fused-core particle which leads to an excellent Peak shape, good Separation, very fast Analysis. Excellent LOQ and Validation data
6. Based on the supporting information, what are the cons/weaknesses of the method?	No cons
7. Any general comments about the method?	From time to time we are asked to analyze Aloe emodin, which is a Degradation product of the aloin. This is neither mentioned in the SMPR nor in the Method.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	absolutely recommended for the purpose descrobed in the SMPR. Fast Methode for a Routine lab with Standard Equipment, with a good LOQ and Validation data.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-30 03:41:48
Name	Darryl Sullivan
E-mail	darryl.sullivan@covance.com
Organization	Covance Laboratories
Title of Method	Determination of Aloin A and Aloin B in Aloe vera Raw Materials and Finished Products by High-Performance Liquid Chromatography: Single-Laboratory Validation
AOAC Candidate Method Number (e.g. ALN-01)	ALN-01
Applicable SMPR	2015.015
Summary:	This is an HPLC method that measures aloin and aloin B in Aloe vera raw materials and finished products. A complete single laboratory validation has been completed on this method.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	This method is applicable to the determination of aloin A and aloin B in Aloe vera leaf juice, dietary supplements and finsihed products.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	This method appears to comply with most if not all of the SMPR requirements.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes - this method used the correct definitions.
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes - this method is complete.

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	yes
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	yes - this paper contains results from a full single laboratory validation (SLV)
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	yes - this paper contains results from a full single laboratory validation (SLV) There is no discussion of a reference material.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	yes - this paper contains results from a full single laboratory validation (SLV)
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	no

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	while the method does not specifically address system suitability, it does contain all of the parameters required for a robust HPLC method.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	N/A
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	This method is written clearly and it is easy to follow and understand.
5. Based on the supporting information, what are the pros/strengths of the method?	This method is well written. It has successfully been through ta complete SLV and the performance was very good.
6. Based on the supporting information, what are the cons/weaknesses of the method?	N/A
7. Any general comments about the method?	no
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	I recommend that this method be considered for AOAC Official First Action status.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-08-01 13:00:33
Name	Jianming Tao
E-mail	jtao@spectrixservices.com
Organization	Spectrix Analytical Services, LLC
Title of Method	Determination of Aloin A and Aloin B inAloe vera Raw Materials and Finished Products by High-Performance Liquid Chromatography: Single-Laboratory Validation
AOAC Candidate Method Number (e.g. ALN-01)	ALN-01
Applicable SMPR	2015.015
Summary:	Authors developed and validated an HPLC method to detect and quantify aloin A and aloin B in Aloe vera raw materials and finished products. It is shown that the described method fits the AOAC SMPR 2015.015 except for collaborative study.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	No. SMPR requires LOQ for aloin A or B at 0.005 ppm. However, the described method has LOQ for aloin A at 0.23 ug/ml (ppm) and for aloin B at 0.21 ug/ml (ppm). The low end of the analytical range of the method would be higher than that of SMPR at 0.01 ppm for aloin A or B, accordingly. Authors did not demonstrate the performance parameters of all ranges described in SMPR for Repeatability, Recovery, and Reproducibility, especially for the low end, 0.01-1 ppm.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	No. The CAS numbers for aloin A and B in the method need to be updated. Acceptable HorRat values should be 0.3-2, and in the section of 'Single-Laboratory Validation (SLV) Parameters indicates 0.1-2.
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	No. Suggestion: General chemical safety measurement should be taken when conducting experiments.

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Collaborative study should be conducted to obtain the Reproducibility.
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	Yes.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	Yes.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	Yes.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	Need include system suitability methods, and further investigation to get lower LOD/LOQ than current ones.

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	No. Need suitability tests including blank check sample, check standard at the midrange point of the analytical range.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	No. The authors did not mention the system suitability tests.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	In revision, authors should include the system suitability tests, collaborative study, and improvement of LOD/LOQ.
5. Based on the supporting information, what are the pros/strengths of the method?	The authors carefully tested the method using AOAC Single-Laboratory Validation (SLV) criteria. The developed method was able to quantitate aloin A and B separately in Aloe vera raw materials and finished products.
6. Based on the supporting information, what are the cons/weaknesses of the method?	The method does not contain system suitability tests. It also has high LOD/LOQ to limit the analytical range.
7. Any general comments about the method?	The method will meet the AOAC SMPR 2015.015 after necessary revision. However, regarding the LOQ, do we really need set the LOQ so low, since the International Science Aloe Council set up the an industry guideline of less than or equal to 10 ppm total aloins at single-strength concentrations to considered safe for ingredients in products intended for oral consumption? The low LOQ means that long and big columns should be used to be suitable for big volume of injections, resulting long running time and big volume of solvent waste. Today, I would like to see UPLC system could be used for the method to save time and reduce solvent waste. UPLC-MS could give analysts extra confidence to identify the aloins.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	Yes. The authors has addressed their method performance characteristics with accuracy, sensitivity, specificity, reproducibility, LOD, LOQ, Lineality, and range of the test method using AOAC guidelines. The method could be used to detect the level of aloin A and B in Aloe vera ingredients and products with a degree of confidence. However, I noticed this validate method was published before AOAC SMPR 2015.015, a proper revision in certain areas should be made per AOAC SMPR 2015.015.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-25 13:13:45
Name	Marina Torres
E-mail	mtorres@latu.org.uy
Organization	LABORATORIO TECNOLOGICO DEL URUGUAY
Title of Method	Determination of Aloin A and Aloin B in Aloe vera Raw Materials and Finished Products by High-Performance Liquid Chromatography: Single-Laboratory Validation
AOAC Candidate Method Number (e.g. ALN-01)	ALN-01
Applicable SMPR	2015.015
Summary:	Solid test materials are extracted using 0.1% (v/v) acetic acid in methanol or 0.1% (v/v) acetic acid in water depending on their Aloin concentration. Extraction is performed using sonication. Liquid test materials only required dilution, or direct injection prior to filtration and HPLC analysis. Separation was achieved using a fused core C18 column in 18 min under isocratic elution conditions. Both Aloin A and Aloin B are quantified using Aloin A calibration curve.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes. The method can quantitate Aloin A and Aloin B separately in aloe vera leaf juice, dry juice ingredients and dietary supplement finished products.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Analytical range, LOD and system suitability do not meet SMPR specifications. For repeatability presented data meet the SMPR but more data is needed. Data for recovery can meet the SMPR or not depending on the interpretation of the paper (see below). Anyway, more data is needed
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes. No hazardous steps in the procedure and small quantities of solvents are used.

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	This SMPR does not state any specific RM.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	This SMPR does not state any specific RM.

4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	1-LOD AND ANALYTICAL RANGE : LOQ target is 0.005 ppm. LOQ (liquid samples): for Aloin A is 0.23 µg/mL (ppm) and for Aloin B is 0.21 g/mL. LOQ (powders & capsules): for Aloin A is 2.3 µg/g (ppm) and for Aloin B is 2.1 µg/g So target LOQ is not meet. This means Analytical Range of SMPR is not meet. 2-SYSTEM SUITABILITY The authors use blank samples because they used them for spiking in order to calculate recovery but they do not show chromatograms of blanks samples. No check standards are used as specified in SMPR. 3-REPEATABILITY Four replicate preparations of each test material were prepared and analyzed on 3 separate days. Supplementary information stated that the values in the paper correspond to intermediate precision values, so for the evaluation I consider the repeatability values of the supplementary information. This parameter was evaluated for: -TM2 – Inner leaf dry juice powder, approximate level 7 ppm, reported RSDr were 3.6 and 2.8%. Both of them were below 11%, so they are OK -TM3 – Nondecolorized leaf dry juice powder, approximate level 25ppm, reported RSDr were 0.83 and 4.3%. Both of them were below 7%, so they are OK -TM5 – Powder capsule, approximate level 7 ppm, reported RSDr were 3.6 and 2.8%. Both of them were below 11%, so they are OK -TM6 – Nondecolorized leaf juice, approximate level 7 ppm, reported RSDr were 3.6 and 2.8%. Both of them were below 11%, so they are OK -TM8 – Nondecolorized inner leaf juice, approximate level 14000 ppm, reported RSDr were 0.85 and 0.51%. Both of them were below 3 %, so they are OK Note: TM6 and TM8 concentration are higher than the maximum value stated in SMPR Analytical Range, but they can show that the technique performs OK at higher values. Concentrations between 25-14000 ppm should be analyzed. Concentrations below 4 ppm should be analyzed too but this is not possible due to LOQ. 4-RECOVERY: Both liquid and solid materials were spiked in triplicate at three levels. Solid samples: The paper states “For the solid matrix recovery study, a commercial spray-dried A. vera powder was spiked to expected concentrations of 4, 40, and 80 µg/mL.” It is not clear if 4, 40, and 80 µg/mL are 4, 40, and 80 µg/g in solid sample (if there is an error in the units) or if these values are concentration in sample extract before injection. If these values are 4, 40, and 80 µg/g in solid sample, the obtained recovery values are all in the range 80-110% so they meet SMPR. But if levels are 4, 40, and 80 µg/mL in the sample extract before injection, this means all Aloin A values meet the SMPR, but some recoveries values for Aloin B do not meet the SMPR. Level 4 recovery for Aloin B at 4 µg/mL (40 ppm in sample) is between 80-110%, so meets SMPR, but levels 40, and 80 µg/mL (that means 400 and 800 ppm in sample) are outside 90-107% (84.41 and 88.06% respectively). Liquid samples: all values meet the SMPR 5-LINEARITY: Seven point calibration curve is used from 0.3 to 50 µg/ml r2 ≥0.999 and when is necessary to use a four calibration curve r2 ≥0.999 too
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	Method does not meet SMPR now. More data would be needed for repeatability and recovery to cover all analytical range of SMPR, but before doing this the method needs to be improved in order to obtain the LOQ required by SMPR

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	The authors use blank samples because they used them for spiking in order to calculate recovery but they do not show chromatograms of blanks samples. Check standards and a control sample must be included. QC used for the method is resolution between isomers only. Only Aloin A is used for calibration for both, Aloin A and Aloin B isomers. It was checked that both isomers have the same response to UV and it is not necessary to use a response factor?
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	No check standards nor control samples.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	It is not very clear why some samples (highly concentrated) are diluted or extracted using methanol but low content samples does not use methanol. It could be good if this is explained because the way of “Preparation of Test Samples” is written is a little bit confuse although the extraction solvent is well explained in the item SOLUTIONS The flow is 1.85 or is a typing error? In item SOLUTIOS (a) it is stated that reference standard diluents is 100% methanol but in item STABILITY OF MIXED STANDARD it is stated that the stability is higher with the addition of 0.1% acetic acid.
5. Based on the supporting information, what are the pros/strengths of the method?	Pros: easy, fast, clean chromatograms. It is better to have a DAD but could be performed with a UV detector.
6. Based on the supporting information, what are the cons/weaknesses of the method?	Method must be improved in order to have lower LOQ.
7. Any general comments about the method?	Good validation data although does not meet this stakeholder panel needs.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	I do not recommend to move forward to First Action. Method must be improved in order to decrease LOD. After this, more data would be necessary in order to cover analytical range stated in SMPR.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-22 16:34:42
Name	tom phillips
E-mail	tom.phillips@maryland.gov
Organization	SCS MDA
Title of Method	Determination of Aloin A and Aloin B in Aloe vera Raw Materials and Finished Products by HPLC: Single Laboratory Validation
AOAC Candidate Method Number (e.g. ALN-01)	ALN-01
Applicable SMPR	SMPR 2015.015
Summary:	An extraction procedure using sonication with an acidified solvent was used for solid test materials while liquid test materials only required dilution, if necessary, prior to filtration and analysis. Separation was achieved using a fused core C18 column in 18 min under isocratic elution conditions allowing for a single analyte (aloin A) calibration curve to quantify both aloins. Adequate chromatographic resolution (Rs >1) was achieved for aloin A and aloin B. The calibration curves for aloin A exhibited coefficients of determination (r2) of >99.9% over the linear range of 0.3–50 µg/mL. The LOD values were 0.092 and 0.087 µg/mL, and LOQ 0.23 and 0.21 µg/mL for aloin A and aloin B, respectively. Repeatability studies were performed on nine test materials on each of 3 separate days, with five of the test materials determined to be above the LOQ having repeatability RSD (RSDr) values ranging from 0.61 to 6.30%. Method accuracy was determined through a spike recovery study on both liquid and solid matrixes at three different levels: low, medium, and high. For both aloins, the recovery in the liquid matrix ranged from 92.7 to 106.3% with an RSDr of 0.15 to 4.30%, while for the solid matrix, the recovery ranged from 84.4 to 108.9% with an RSDr of 0.23 to 3.84%.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Partially

4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	No
1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	No supporting documents submitted
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	Spikes were used, but no standardized reference aloin containing products were used.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	Spikes were used, but no standardized reference aloin containing products were used.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	Yes, except for LOQ. The method LOQ is approximately 2.2 ppm, the SMPR LOQ is 0.005 ppm (5 ppb).

1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No supporting documents submitted.
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Blanks only. Thee was no control sample used. There was a QC standard used at 25 ppm.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	Only the blank was used, there is no mention of the QC standard being used in the discussion. This is due to no supporting documentation being submitted.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes, the method is very straight forward.
5. Based on the supporting information, what are the pros/strengths of the method?	Pros: small sample size, small amount of organic solvent used. Cons: Small samples size can lead to high variablity if the product is not homogenous, detection limit is high due to small sample size.
6. Based on the supporting information, what are the cons/weaknesses of the method?	Pros: small sample size, small amount of organic solvent used. Cons: Small samples size can lead to high variablity if the product is not homogenous, detection limit is high due to small sample size.
7. Any general comments about the method?	The issue with the separations method is that it is in fact a gradient and not isocratic according to Table 1 and in the solutions section, it shows Mobile Phase A and B. This also indicates that it is a gradient.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	no, it needs additional work on meeting the SMPR's

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AOAC Stakeholder Panel on Dietary Supplements Expert Review Panel

ALN-02

Method Title: Determination of Aloin A, Aloin B, and Aloe-emodin in Raw Materials and Finished Products by HPLC

Submitted by: Steven Dentali

Submitted by Email: stevend@herbalife.com

Enclosures: 1

Submitter notes: Method submitted for Aloin in Aloe Vera Ingredients and Products. Method validation provided as supporting document. Please let us know if further information is requested.

Link to Method: http://griegler-aoac-org.cld.bz/ALN-02
Password: aoacaoac
Primary Reviewer: Holly Johnson

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-28 14:38:49
Name	Nour Eddine ES-SAFI
E-mail	nouressafi@yahoo.fr
Organization	Mohammed V University in Rabat
Title of Method	Determination of Aloin A, Aloin B and Aloeemodin in Raw Materials and Finished Products by HPLC
AOAC Candidate Method Number (e.g. ALN-01)	ALN-02
Applicable SMPR	AOAC SMPR 2015.015
Summary:	The proposed methods presents results dealing with the quantitative analysis of aloin A, aloin B and aloe-emodin in raw materials and finished products by RP HPLC coupled to a PDA detector.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	* Analytical range: 0.01 - 0.5 ppm (SMPR: 0.01-100 for finished products and 0.01-12500 for raw materials) * LOQ: 0.02 ppm (SMPR: 0.005 for aloin A and B in both finished and raw material) * LOD: 0.01 ppm * Repeatability: RSDs were less than 10 % and were in agreement with the SMPR tables. * Recovery: 82.9-100.1 % (Aloin A) and 85.5-89.5 % (aloin B) which are in agreement with the SMPR which values range from 60-115 % in the corresponding concentrations. * Reproducibility: not conducted
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes, the definitions specified in the SMPR were used in the proposed method. The authors used
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	No
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	No
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	Reproducibility is missing
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Yes, the method contains system suitability tests through the injection of standards.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	Yes
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes
5. Based on the supporting information, what are the pros/strengths of the method?	The proposed method seems to be simple robust and the obtained results seem to be correct.
6. Based on the supporting information, what are the cons/weaknesses of the method?	identification and purity of the quantified analytes.
7. Any general comments about the method?	no
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	I think that the proposed method could be (after discussion with the ERP members) recommended to be adopted as a first action.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-15 19:07:35
Name	Quanyin Gao
E-mail	quanying@herbalife.com
Organization	Herbalife
Title of Method	Aloins
AOAC Candidate Method Number (e.g. ALN-01)	ALN-02
Applicable SMPR	2015.015
Summary:	The test method of HPLC quantitative analysis of Aloins using UV detector is easy to implement. The method is sensitive (LOD of 10 ppb and LOQ at 20 ppb), specific, accurate, rugged and precise.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	The method support the applicability of SMPR
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	The method meets SMPR
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	The definitions are apporpriate.
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	the method contained appropriate precautions.
1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Derfinitions are appropriate.

2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	The method meets the SMPR performance requirements.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	The method meets the performance requirements using hte ref materials.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	The validation report demonstrated method performance.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No additional statement required.
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	System suitability parameters are present.

3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	The system suitability control is adequate.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	The method is written clearly.
5. Based on the supporting information, what are the pros/strengths of the method?	The strength of the method is that many commercial product formulations have been tested during method validation.
6. Based on the supporting information, what are the cons/weaknesses of the method?	Not known of.
7. Any general comments about the method?	The method is ready to be adopted in industry.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	Yes.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-30 18:02:20
Name	Phil Koerner
E-mail	philk@phenomenex.com
Organization	Phenomenex
Title of Method	Determination of Aloin A, Aloin B and Aloe- emodin in Raw Materials and Finished Products by HPLC
AOAC Candidate Method Number (e.g. ALN-01)	ALN-02
Applicable SMPR	2015.015
Summary:	Gradient HPLC-UV method for determination of Aloin A, Aloin B, and Aloe-emodin in raw materials and finished products.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	yes; with addition of aloe-emodin which is not required by SMPR
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	should refer user to MSDS for reagents used; commonly used reagents for typical HPLC lab
1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	yes

2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	yes; LOD and LOQ determination based on signal to noise
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	yes;
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	yes
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	no
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	yes; however for gradient HPLC method would prefer to see resolution used for system suitability vs. efficiency also tailing factor NMT 2.0 is quite high for a gradient method; data to support this is meaningful value to use?

3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	yes
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	yes
5. Based on the supporting information, what are the pros/strengths of the method?	relatively straight forward gradient HPLC method; if aloe-emodin not required to quantitate, then overall analysis time could be reduced by adjusting gradient times
6. Based on the supporting information, what are the cons/weaknesses of the method?	time, but that is due to extended scope for this method vs. SMPR requirements
7. Any general comments about the method?	no other comments
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	yes; method meets requirements per SMPR 2015.15 for Aloin A and Aloin B

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-31 22:15:13
Name	charles metcalfe
E-mail	laptop@calabs.us
Organization	custom analytics
Title of Method	Determination of Aloin and Aloe Emodin in Raw Materials and Finished Prodcuts by HPLC
AOAC Candidate Method Number (e.g. ALN-01)	ALN-02
Applicable SMPR	2015.015
Summary:	Analysis of Aloins and Aloe Emodin by HPLC
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Non hazardous method or chemicals.
1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes

2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	Yes
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	Yes
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	Yes
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Yes

3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	No blank check, no control sample
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes
5. Based on the supporting information, what are the pros/strengths of the method?	Very good reproducibility at the 100 ppb level. Validation report states a 10 ppb spike but a 10 ppm recovery. Which is a typo?? Page 6/7/8 of 21.
6. Based on the supporting information, what are the cons/weaknesses of the method?	Only con is the amount of sample prep required. Dilute and shoot is the best way to minimize sample prep errors. Extraction, evaporation, reconstitution is alot of places for error. Plus requires a fume hood to handle the evaporation of the solvents.
7. Any general comments about the method?	Good sensitive and reproducible method. In my experience I do not see any less matrix interference compared to a dilute and shoot method.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	Yes

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-20 06:15:11
Name	Klaus Reif
E-mail	klaus.reif@phytolab.de
Organization	PhytoLab GmbH
Title of Method	Determination of Aloin A, Aloin B and Aloeemodin in Raw Materials and Finished Products by HPLC
AOAC Candidate Method Number (e.g. ALN-01)	ALN-02
Applicable SMPR	2015.015
Summary:	The method is applicable for the Determination of aloin A, aloin B and Aloe emodine in raw materials as well as finished products by Standard HPLC equipment
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	YES
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	YES
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	YES
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	NO All reagents and chemicals should be referred to the corresponding safety data sheets
1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	YES

2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	No reference materials available
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	No reference materials available
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	NO; the required LOQ of 5 ppb is not fulfilled by this method. LOD = 10 ppb LOQ = 20 ppb
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	NO
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	YES

3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	NO. SST is described in the SOP but in the Validation part no results or data referring to the SST are reported
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	yes
5. Based on the supporting information, what are the pros/strengths of the method?	modern HPLC-equipement, Aloe emodine could be detected within the same HPLC runs. Complete and excellent Validation data
6. Based on the supporting information, what are the cons/weaknesses of the method?	the run time is very Long and could be accelareted
7. Any general comments about the method?	Fully recommend this method as Aloe emodine is included (though the SMPR doesn't require this compound. But in the past we had several request of customers to include this into the method. The SMPR requires a LOQ of 5 ppb which is not fulfilled (20 ppb). For finished products I usually saw specifications of <100 ppb. So a LOQ of 20 ppb would be sufficient. A really good Routine method applicable in a Standard Routine lab.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	s. 7

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-30 05:19:46
Name	Darryl Sullivan
E-mail	darryl.sullivan@covance.com
Organization	Covance Laboratories
Title of Method	Determination of Aloin A and Aloin B and Aloe-emodin in Raw Materials and Dietary Supplements using HPLC
AOAC Candidate Method Number (e.g. ALN-01)	ALN-02
Applicable SMPR	2015.015
Summary:	this method measures Aloin A, Aloin B, and Aloe-emodin in raw materials and dietary supplements using HPLC with UV detection.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	yes
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	It appears that this method meets the majority of the requirements in the SMPR
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	yes
1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	yes

2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	There is no discussion regarding reference materials
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	yes
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	yes
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	no
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	yes

3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	yes
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes, this method is well written and easy to follow.
5. Based on the supporting information, what are the pros/strengths of the method?	The method is straightforward and appears to perform well.
6. Based on the supporting information, what are the cons/weaknesses of the method?	n?A
7. Any general comments about the method?	no
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	I recommend that this method be given additional consideration by the ERP. I am not sure if I see enough date to make a recommendation for First Action status - but the ERP should consider this method.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-28 11:26:30
Name	Marina Torres
E-mail	mtorres@latu.org.uy
Organization	LATU
Title of Method	Determination of Aloin A, Aloin B and Aloeemodin in Raw Materials and Finished Products by HPLC
AOAC Candidate Method Number (e.g. ALN-01)	ALN-02
Applicable SMPR	2015-015
Summary:	Solid samples are extracted in water using vortex. Reagent alcohol and saturated sodium chloride are added to the water extract or liquid samples in order to facilitate extraction of Aloins from water extract to organic layer. Organic layer is evaporated to dryness and the dried sample residue is dissolved in methanol/water (60/40). Separation was achieved using a C18 Phenomenex Synergi Hydro-RP column under gradient elution conditions.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes. The method can quantitate Aloin A and Aloin B separately in Aloe Vera raw materials and finished products.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Analytical range does not meet SMPR specifications. For repeatability and recovery presented data meet the SMPR. LOQ (20 ppb in sample extract that means 10 ppb in sample) is slightly higher than the SMPR requirement of 5 ppb in sample.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes. No hazardous steps in the procedure and small quantities of solvents are used.

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	This SMPR does not state any specific RM.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	This SMPR does not state any specific RM.

4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	1-ANALYTICAL RANGE: this technique is for low concentration products and do not meet SMPR requirement. 2-LOQ: is slightly higher than the SMPR requirement of 5 ppb in sample. 2-SYSTEM SUITABILITY Blank samples are used and show no interference at the retention times of Aloin A and Aloin B. Check standard at midrange point of the analytical range is used but no check standard at the lowest point. The Correlation Coefficient (R) for the Aloin A and Aloin B curves are not less than 0.998. Theoretical plate count: no less than 10,000 for Aloin and Aloin B Tailing factor: Not more than 2.0 for Aloin A and Aloin B. A control sample must be included. It should be good to include resolution between Aloin A and B requirement. 3-REPEATABILITY Six replicates of four samples (two of them were no detected) were prepared and analyzed. All data meet the SMPR requirements. 4-RECOVERY: Both liquid and solid materials were spiked in triplicate at three levels. All values meet the SMPR requirements. 5-LINEARITY: Six point calibration curves were used from 10 to 500 ppb r2 ≥0.9999
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	Method does not meet SMPR due to Analytical Range. The technique is for low concentration products only.
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Blank samples are used. Check standard at midrange point is used. The Correlation Coefficient (R) for the Aloin A and Aloin B curves are not less than 0.998. Theoretical plate count: no less than 10,000 for Aloin and Aloin B Tailing factor: Not more than 2.0 for Aloin A and Aloin B.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	No control samples, no check standard at lowest point.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes, the method is written very clearly. Validation report is not so clear: I did not understand what the notes in pages 3/21 and 4/21 mean. “Aloin found” in tables VII, VIII,IX,X,XI and XII is expressed in ppm but must be ppb.

5. Based on the supporting information, what are the pros/strengths of the method?	Pros: easy method, clean chromatograms. It is better to have a DAD but could be performed with a UV detector. The method has the possibility to measure Aloe-Emodin too.
6. Based on the supporting information, what are the cons/weaknesses of the method?	The weakness for this stakeholder is that the Analytical Range does not meet SMPR
7. Any general comments about the method?	Very good method, good validation data. Method for low concentration products only.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	I do not recommend to move forward to First Action because does not meet Analytical Range. Method could be improved in order to analyze products of higher concentration. Note: Maybe this method could be used with ALN01 to cover analytical range of SMPR

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-22 17:07:01
Name	tom phillips
E-mail	tom.phillips@maryland.gov
Organization	SCS MDA
Title of Method	Determination of Aloin A, Aloin B and Aloeemodin in Raw Materials adn Finished Products by HPLC
AOAC Candidate Method Number (e.g. ALN-01)	ALN-02
Applicable SMPR	SMPR 2015.015
Summary:	The method utilizes extraction of a small weight of powdered material and about a gram of liquid material with a two phase extraction utilizing ethyl acetate and salt to achieve separation of layers. The sample is extracted 2x with ethyl acetate and the layers combined. The extract is evaporated to dryness and brought to volume. The extract then is analyzed by HPLC DAD or UV.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Yes, except for the LOQ. The LOQ is 0.100 ppm and does not meet the SMPR requirement of 0.005 ppm
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	No, there is no safety statement or cautionary statements.

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	yes
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	No, there were spikes only, no standard reference materials were used.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	No, there were spikes only, no standard reference materials were used.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	no, there needs to be reproducibility and repeatablilty work done in actual samples and not just spikes.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	no

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	yes
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	yes
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	yes
5. Based on the supporting information, what are the pros/strengths of the method?	Pros: small sample weight,, use of small amounts of organic solvents. Cons: small sample weight, steps are somewhat confusing when referencing previous steps. length of hplc run
6. Based on the supporting information, what are the cons/weaknesses of the method?	Pros: small sample weight,, use of small amounts of organic solvents. Cons: small sample weight, steps are somewhat confusing when referencing previous steps. length of hplc run
7. Any general comments about the method?	The method has potential, more data is needed from actual products supporting the reproducibility, etc.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	no, the method does not meet all the SMPR requirements.

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AOAC Stakeholder Panel on Dietary Supplements Expert Review Panel

AOAC Candidate Method #CIN-01

Identification of Selected *Cinnamomum* spp.Bark in Dietary Supplement Raw Materials and/or Finished Products - Gas Chromatography with Flame Ionization Detection After Hydrodistillation

- Author(s): Myron Sasser, Gary Jackoway, Craig Kunitsky
- Submitted by: Craig Kunitsky, Midi
- Enclosures: 2
- Submitter notes: The main contact for method questions and feedback is Gary Jackoway.

- RESUBMISSION 7/7 Notes:
  Attached please find the updated MIDI Cinnamon Method.

  The major changes are:
  - Addition of C. ramulus to the species identification.
  - Alternative automated peak naming using MIDI Sherlock software.
  - Alternative pattern matching species identification using MIDI Sherlock software.

Link to Method: http://griegler-aoac-org.cld.bz/CIN-01
Password: aoac6

Primary Reviewer: Tom Phillips

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-08-01 15:17:30
Name	tom phillips
E-mail	tom.phillips@maryland.gov
Organization	SCS MDA
Title of Method	Identificaton of Selected Cinnamomum spp. Bark in Dietary Supplement Raw Materials and/or Finished Products
AOAC Candidate Method Number (e.g. ALN-01)	CIN-01
Applicable SMPR	2015.010
Summary:	Volatile oils from Cinnamomum spp. are extracted into toluene using hydrodistillation.The volatile oil extract is then analyzed by gas chromatography-flame ionization detection GC-FID). The Cinnamomum spp. are identified using a series of predetermined tests (hierarchical decision tree) and comparison of these results to known species. Interference compounds from potentially cross-reactive substances are excluded from the calculations. Analysis can be done manually, or automatically using MIDI, Inc.'s Sherlock Supplement Analysis software package.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	No, C. tamala was not analyzed in the method
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	No, Dietary ingredient was not used
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	There is no safety statement.

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	no, Dietary ingredients is not used.
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	No, there is no indication of replicates for the authenticated cinnamon materials.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	No, there is no indication of replicates for the authenticated cinnamon materials.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	n/a
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	There are no precautionary statements present in the method. The method involves distillation and the use of flammable solvents, as well as electrical and heat sources.

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	The method uses alkane standards to determine a retention index similar to the Kovats retention index. The section of E.a needs to be rewritten. The standards are made in a 500 ml VF and not brought to volume. An aliquot of 250-mls of hexane are added. Considering 7 of the 11 compounds in Section H are commercially available, they should have been used in the std mix to verify the retention times.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	No. Just a summary, no report from the software on how the numbers were evaluated.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes
5. Based on the supporting information, what are the pros/strengths of the method?	clean mixture of volatile components trapped in the toluene layer. The short run time would allow approximately 4 samples per hour to be analyzed on the instrument
6. Based on the supporting information, what are the cons/weaknesses of the method?	The use of FID instead of MS to identify the components of a mix. This could lead to misidentification of components. There are many plant materials that have linalool, cineole, copaene, coumarin, curcumene in them that can have potential interferences in the identification. Retention index is only useful if there is nothing but cinnamon derived material in the dietary supplements.
7. Any general comments about the method?	Good method, there is no indication of the necessary 33 replicates representing both Table I and II.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	No, there is no data on replicates and none of the ingredients in the exclusivity panel were analyzed.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-29 12:45:19
Name	Martha Jennens
E-mail	martha.jennens@covance.com
Organization	Covance
Title of Method	Identification of Selected Cinnamomum spp. Bark in Dietary Supplement Raw Materials and/or Finished Products
AOAC Candidate Method Number (e.g. ALN-01)	CIN-01
Applicable SMPR	2015.010
Summary:	Volitile oils from Cinnamomum spp. are extracted into tolulene using hydrodistillation. The volatile oil extract is then analyzed by gas chromatography-flame ionization detection. The Cinnamomum spp. are identified using a series of predetermined tests and comparison of these results to known species.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes, it supports the SMPR with identification of Cinnamomum spp.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Yes, the method supports the SMPR
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes, the definitions from the SMPR are used in the method
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	No precautions are noted in the method

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes, the definitions are used and applied in the method.
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	The supporting data provided shows that five of the six selected Cinnamomum spp. from the SMPR were used.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	The supporting data demonstrates that the method performs according to the SMPR requirements for identification. There is no supporting data with the nontarget compounds.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	Yes, the method meets the method performance requirements in the SMPR with the exception of the exclusivity panel.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	Safety/handling of reagents, etc.

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Yes, the system suitability is very good and clearly explains the process of what steps need to be taken if there is a failure.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	The SPMR system suitability requirements are met.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes, the method is clearly written
5. Based on the supporting information, what are the pros/strengths of the method?	The method is clear and provides way to differentiate and quantitate Cinnamom spp.
6. Based on the supporting information, what are the cons/weaknesses of the method?	There were no tests done with the exclusivity panel
7. Any general comments about the method?	Good method. Need to see data with matrices from the exclusivity panel.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	Yes, if exclusivity data can be performed prior to first action.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-30 04:09:03
Name	Darryl Sullivan
E-mail	darryl.sullivan@covance.com
Organization	Covance Laboratories
Title of Method	Identification of Selected Cinnamomum spp. Bark in Dietary Supplement Raw Materials and / or Finished Products
AOAC Candidate Method Number (e.g. ALN-01)	CIN-01
Applicable SMPR	2015.010
Summary:	Volatile oils from Cinnamomum spp. are extracted into toluene using hydro-distillation. This oil is then analyzed using gas chromatography with flame ionization detection. The results are compared with those of known species of Cinnamomum.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	The applicability of this method appears to align with the SMPR.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	This method appears to meet the majority of the SMPR requirements. I do not find any data regarding exclusivity challenge studies.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	The definitions appear to be appropriate.
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	The method is complete and contains all of the appropriate precautions.

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	yes
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	The method contains the majority of the information required in the SMPR - but no exclusivity data are available.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	The SMPR requires a minimum of 33 replicates representing the selected Cinnamomum species. This data will need to be collected.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	Additional data replicates are required for exclusivity and exclusivity.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	no

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	yes
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	yes
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	The method is well written. It is however a complex procedure, and it may need some additional language.
5. Based on the supporting information, what are the pros/strengths of the method?	This method appears to have a scientifically valid approach to ID testing.
6. Based on the supporting information, what are the cons/weaknesses of the method?	This method has not been through exclusivity challenges.
7. Any general comments about the method?	This method should be challenged through a complete single lab validation.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	I recommend that this method be given additional consideration. I would like to see SLV data that includes exclusivity data, before I would recommend this method for First Action status.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-08-01 00:02:21
Name	Joseph Zhou
E-mail	josephzhou@sunshinevillehp.com
Organization	Sunshineville Health Products, Inc
Title of Method	Identification of Selected Cinnamomum spp.Bark in Dietary Supplement Raw Materials and/or Finished Products - Gas Chromatography with Flame Ionization Detection After Hydrodistillation
AOAC Candidate Method Number (e.g. ALN-01)	CIN-01
Applicable SMPR	Identification of Selected Cinnamomum spp.Bark in Dietary Supplement Raw Materials and/or Finished Products
Summary:	Volatile oils from Cinnamomum spp. are extracted into toluene using hydrodistillation.The volatile oil extract is then analyzed by gas chromatography-flame ionization detection (GC-FID). The Cinnamomum spp. are identified using a series of predetermined tests (hierarchical decision tree) and comparison of these results to known species. Interference compounds from potentially cross-reactive substances are excluded from the calculations. Analysis can be done manually, or automatically using MIDI, Inc.'s Sherlock Supplement Analysis software package.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	The identification method is clearly presented. But no test was performed on Cinnamon tamala, one of the 6 selected cinnamomum spp. bark required by SMPR. Also no data is available to meet SMPR Method Performance Requirements; no data on Exclusivity as required by SMPR.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	The method as presented looks a good method. The description is detail and clear. But the publication does not show any essential data to support the method development.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Again, the method is clearly presented, but no data is presented to support the method.
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes, the method is written very clearly.

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Each step of the method is clearly presented, but without any supporting data.
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	No information is available to show if the method meets the SMPR method performance requirements.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	No information is available to show if the method meets the SMPR method performance requirements.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	No information is available to show if the method meets the SMPR method performance requirements.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	The method is well written. The only problem is no data to support the method.

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Yes, the method contains system suitability tests.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	The system suitability tests are well designed in the method.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	The method is written clearly and concisely. But no supporting data is available in the same publication.
5. Based on the supporting information, what are the pros/strengths of the method?	Based on its description, this method looks a good method. But the authors should also present the supporting data in order for us to determine if the method meets the SMPR requirements.
6. Based on the supporting information, what are the cons/weaknesses of the method?	The same as above.
7. Any general comments about the method?	It looks a very good method, but AOAC should request the authors to submit the method development supporting data.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	This method as presented looks a very good ID method. It was written in a very professional way. But since no supporting method development data is presented, it is not ready to be adopted as a First Action. AOAC should contact the authors to submit their supporting data.

The Scientific Association Dedicated to Analytical Excellence®

AOAC Stakeholder Panel on Dietary Supplements Expert Review Panel

AOAC Candidate Method #TEA-01

Analysis of Theanine in Tea (Camellia sinensis) Dietary Ingredients and Supplements by High-Performance Liquid Chromatography with Post-Column Derivatization: Single Laboratory Validation

- Author(s): Maria Ofitserova, Sareeta Nerkar
- Submitted by: Maria Ofitserova, Pickering Labs
- Enclosures: 0
- Submitter notes: Tables and chromatograms are grouped at the end of the document.

Link to Method: http://griegler-aoac-org.cld.bz/SPDS-ERP-TEA-01

Password: 35aoac

Primary Reviewer: Melissa Phillips

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-28 16:42:40
Name	Melissa Phillips
E-mail	melissa.phillips@nist.gov
Organization	NIST
Title of Method	Analysis of Theanine in Tea (Camellia sinensis) Dietary Ingredients and Supplements by High- Performance Liquid Chromatography with Post-Column Derivatization: Single Laboratory Validation
AOAC Candidate Method Number (e.g. ALN-01)	TEA-01
Applicable SMPR	2015.014
Summary:	This method is presented for determination of theanine in green tea containing dietary ingredients and supplements. The method utilizes simple sonication extraction in LiCitrate buffer and cation exchange chromatography with post-column ninhydrin derivatization before absorbance detection. Tested products include powders, liquids, tablets, capsules, softgels, and gelcaps. Method did not address gummies or chewables.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	The method only addresses the determination of theanine. Method does not address determination of catechins, methyl xanthines, or theaflavins.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Considering only theanine: Analytical range stated in SMPR: 10-100,000 ppm. Lowest calibration level in method was 0.5 ug/mL or ~500 ppm. Lowest product tested was 41 ppm. Method states to use 0.1 to 1.0 g portions of sample (does this cover the range of samples concentrations specified in the SMPR?). LOQ stated in SMPR: <5 ppm. LOQ calculated in method: 3 ppm. Recovery stated in SMPR was met for all conditions (considering only total recovery). If marginal recovery is considered, method is outside spec for liquid product (109% at 575 ppm). 80-110% for 10-50 ppm 90-107% for 51-500 ppm 95-105% for 501-10,000 ppm RSDr stated in SMPR was met for all conditions. <7% for 10-50 ppm <5% for 51-10,000 ppm Tested products include powders, liquids, tablets, capsules, softgels, and gelcaps. Method did not address gummies or chewables.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes.
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes, very clear section on safety included.

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes.
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	Yes. 3 NIST SRMs were used for demonstration of accuracy. Determination of theanine in all 3 materials was within uncertainty of the assigned values.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	Yes. 3 NIST SRMs were used for demonstration of accuracy. Determination of theanine in all 3 materials was within uncertainty of the assigned values.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	The method only addresses the determination of theanine. Method does not address determination of catechins, methyl xanthines, or theaflavins. The method is very specific for theanine and it does not make sense to extend the applicability to the other analyte groups.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No.

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Yes. A blank check sample could be added to comply with SMPR. Consider modifying the statements about retention times to include an acceptable % deviation with a specified number of replicate injections.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	Yes.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes. I would add more guidance under the section for sample preparation to assist in choice of sample size and extraction volume. Something like "Choose sample size corresponding to approximately X mg of theanine".
5. Based on the supporting information, what are the pros/strengths of the method?	The method is simple and straightforward (sonication extraction, chromatography, detection). The method has demonstrated accuracy via use of reference materials as well as spike/recovery studies. The method has demonstrated adequate precision as specified in the SMPR. The method has adequate linearity, LOQ, and LOD as stated in the SMPR.
6. Based on the supporting information, what are the cons/weaknesses of the method?	High marginal spike/recovery for liquid green tea product (108.7%). The post-column reactor may be considered "specialized equipment". Unknown how method would respond to gummies/chewables (2 difficult matrices). Sonication extraction might not be sufficient. Ruggedness tests showed sensitivity of the method to the ratio of sample mass to extraction solution volume and also post-column reactor temperature. Adding a statement to the sample preparation section can assist with the selection of appropriate sample size and extraction volume, to reduce likelihood of such issues. In addition, a statement could be added to the apparatus section regarding calibration of the post-column reactor temperature.
7. Any general comments about the method?	Very good method for determination of theanine. While it doesn't meet the full applicability of the SMPR, it is promising as a method for theanine and could be used in conjunction with another method for the other analyte classes.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	The method is a strong candidate, but without understanding of how the method will behave with gummies and chewables (2 difficult matrices that were specifically indicated as important by the working group), I do not recommend that this method is adopted as first action at this time. I recommend that the authors conduct additional studies using gummy and chewable supplements and resubmit, as the method has demonstrated potential.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-27 17:04:10
Name	Phil Koerner
E-mail	philk@phenomenex.com
Organization	Phenomenex
Title of Method	Analysis of Theanine in Tea (Camellia sinensis) Dietary Ingredients and Supplements by High- Performance Liquid Chromatography with Post-Column Derivatization: Single Laboratory Validation
AOAC Candidate Method Number (e.g. ALN-01)	TEA-01
Applicable SMPR	2015.014
Summary:	Analysis of Theanine in Tea (Camellia sinensis) Dietary Ingredients and Supplements by High- Performance Liquid Chromatography with Post-Column Derivatization
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	yes

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	single lab validation data only
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	yes
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	yes
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	Theanine only
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	no

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	yes, but would like to see a sample blank for comparison
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	yes
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	yes
5. Based on the supporting information, what are the pros/strengths of the method?	1. specificity for Theanine 2. meets SMPR requirements for Theanine (LOQ, analytical range)
6. Based on the supporting information, what are the cons/weaknesses of the method?	1. Single source (Pickering Labs) of mobile phase (Li-citrate buffer), cation-exchange column, and post-column derivatization reagent 2. Post-column derivatization system - only one from Pickering demonstrated; not sure what other options are out there for use and if they would be similar enough to generate equivalent results 3. analysis time (ca. 62 minutes injection to injection) 4. did not see sample blank injections; in particular interested to see if any co-elution with ISTD (L-norleucine) in some of the samples where there appear to be other small peaks eluting nearby 5. method applicable to Theanine only; other analytes of interest in Tea will require a separate method (but don't see how this can be avoided) 6. identification of potential interferences from various sample matrices eluting near ISTD (e.g. in green tea softgels)?
7. Any general comments about the method?	post-column derivatization does add some complexity and potentially impact detection limits; however, method results appear to suggest this is not a limitation here
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	Yes Need multiple lab date to demonstrate how this method performs in unaffiliated lab environment in order to demonstrate ease of use, reproducibility, and applicability on different post column derivatization systems

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-13 06:40:10
Name	Klaus Reif
E-mail	klaus.reif@phytolab.de
Organization	PhytoLab GmbH & Co. KG
Title of Method	Determination of Catechins and Caffein in Camillia sinensis Raw Materials, Extracts and Dietary Suppelements by HPLC-UV
AOAC Candidate Method Number (e.g. ALN-01)	TEA-01
Applicable SMPR	2015.014
Summary:	The method is used to determine 7 catechins plus caffein in green tea, extract and dietary supplements containing green tea extract.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	No, the analytes Theobromin, theophyllin, theaflavins and theanin is missing. I guess Theobromin and theophyllin could be detected with this method. For Theaflavins and Theanin two different methods will be necessary
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	NO "regarding precautions refer to the corresponding safety data sheets for each individual reagent"
1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes

2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	SRM 3257 is not listed under materials in the experimental section.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	In principle yes, though several Validation Parameters doesn't fulfill teh requirements of the SMPR
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	In principle yes, though several Validation Parameters doesn't fulfill teh requirements of the SMPR
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	already answered
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	yes

3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	blank injection is missing, check Standards at different concentrations are described.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	yes
5. Based on the supporting information, what are the pros/strengths of the method?	definitely separation power and Speed.
6. Based on the supporting information, what are the cons/weaknesses of the method?	Methyl xanthenes (besides caffein) are missing. Gallic acid is missing though it maybe important. The method has been applied only for green tea and not for fermented black tea.
7. Any general comments about the method?	Excellent Method for green tea (and products). The method should also be tested for black tea. Usually a stabilizing solution (like in the ISO-method) should be used, to the instability of some catechins in solution. Some of the Validation Parameters doesn't fulfill the requirements of the SMPR's, e.h. recovery rate for caffeine only 78.7% for a dietary Supplement.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	The method could be recommended (because of speed and resolution) after having tested for black tea as well and including theobromine and theaphyllin like it is required by the SMPR. Some of the Validation data should be repeated or verified by the applicants.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-08-01 04:13:16
Name	Aniko Solyom
E-mail	asolyom@gaasanalytical.com
Organization	GAAS Analytical
Title of Method	Analysis of theanine in tea (Canella sinensis) dietary ingredients and supplements by HPLC with post column derivatization:SLV
AOAC Candidate Method Number (e.g. ALN-01)	TEA-01
Applicable SMPR	2015.014
Summary:	The method is for the analysis of theanine in various dietary supplements and ingredients. It uses lithium citrate buffer (pH 2.2) for the extraction of theanine from the matrices, followed by cation exchange chromatography, post-column derivatization with ninhydrin reagent and detection at 570 nm.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	The SMPR is for the quantitatiive determination of catechins, methyl xanthenes, theaflavins and theanine. This method adresses only the determination of theanine, but different methods were allowed for each class of analytes, so the applicability of the method partially supports the applicability of the SMPR. applicabil
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	The concentration range of the samples covered 3 ranges out of the 5 defined in the SMPR. In these ranges the method satisfies the requirement stated in the SMPR, with a few exception. (Lowest level of theanine, liquid extract formulation and gelcaps)
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	Yes, three SRM-s were used throughout the study: SRM3254, SRM3255 and SRM3256
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	Yes.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	The method is only for the quantitation of theanine; the other analytes specified in the SMPR were not studied.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	System suitability test is not described in the method. The presented chromatograms show good baseline separation in the selected matrices. The authors used L-theanine from Sigma-Aldrich as the reference standard, but chromatograms of the standards and the calibration curve is not included in the submission.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	Indirectly, in tables
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes
5. Based on the supporting information, what are the pros/strengths of the method?	Pros: - relatively simple sample preparation - applicable for different matrices without major modification
6. Based on the supporting information, what are the cons/weaknesses of the method?	Cons: - requires post column derivatization set-up, that is not very common in dietary supplement labs
7. Any general comments about the method?	Propose for further consideration
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	I would like to see some additional data, but generally it is a promising method for the determination of theanine.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-30 04:59:50
Name	Darryl Sullivan
E-mail	darryl.sullivan@covance.com
Organization	Covance Laboratories
Title of Method	Analysis of Theanie in Tea Dietary Supplements and Supplements bu High Performance Liquid Chromatography with Post Column Derivitization: Single Laboratory Validation
AOAC Candidate Method Number (e.g. ALN-01)	TEA-01
Applicable SMPR	2015.014
Summary:	This method uses a buffer extraction followed by cation-exchange chromatography. post column reaction with Ninhydrin reagent and UV/Vis detection.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	This method meets the SMPR applicability for Theanine.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	The analytical method appears to meet the SMPR for Theanine.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	yes

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	yes
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	yes - this study used three NIST reference materials
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	yes
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	yes
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	no

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	yes
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	yes
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	yes - this method is well written
5. Based on the supporting information, what are the pros/strengths of the method?	This method appears to be rugged and performed well during the SLV study.
6. Based on the supporting information, what are the cons/weaknesses of the method?	This method uses post column dervitization with Ninhydrin which requires some unique instrumentation which may not be available in labs that are not familiar with amino acid analysis.
7. Any general comments about the method?	no
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	I recommend that this method be considered for AOAC Official First Action status for the analysis of Theanine.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-30 13:08:23
Name	Kurt Young
E-mail	kurt.young@nutramfg.com
Organization	GNC/Nutra Manufacturing
Title of Method	Determination of Theanine in Tea
AOAC Candidate Method Number (e.g. ALN-01)	TEA-01
Applicable SMPR	2015.14
Summary:	This method utilizes ionic exchange HPLC and post-column derivitization with ninhydrin to determine the non-proteinogenic amino acid, theanine in tea, tea extracts and tea supplements. The method utilizes a lithium citrate buffer solution as a cation-exchange mobile phase component and as a sample extractant. Nor-leucine is used as an internal standard. Post-column reaction with ninhydrin at 130 deg. C is followed by UV-Vis detection at 570nm
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	It supports the applicability of the theanine analysis portion of the SMPR. Theanine is the only amino acid constituent on the list of analytes for the SMPR, thus necessitating a separate analytical method.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Yes, it meets all of the criteria
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes, they are appropriately used.
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	The method contains a section detailing the safety and other precautions regarding reagent and instrumentation use.

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes.
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	Yes. There is ample data to support the method requirements for reproducibility and accuracy.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	Yes. The method performed within requirements for the three SRM's referenced in the SMPR.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	Yes. There was one product that gave a slightly higher result than that for the upper limit for recovery %, but this was most likely due to the non-uniformity of the product.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	There was an indication by the authors of the method that the recovery specifications from the SMPR were more stringent than those found in AOAC dietary supplement guidelines.

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Yes, it contains suitability tests applicable to the SMPR.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	Yes.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes, the method is written in clearly and concisely, and thoroughly elucidates the necessary steps and materials needed.
5. Based on the supporting information, what are the pros/strengths of the method?	The pros of this method are that it utilizes a rugged, precise method that has been a methodology of choice for amino acid analysis. The post-column derivatization step eliminates the problems associated with pre-column derivatization.
6. Based on the supporting information, what are the cons/weaknesses of the method?	The cons/weaknesses are those that are encountered with the general methodology/instrumentation used; long run times, staining from ninhydrin reagent, use of instrumentation/columns/buffers that are only useful for a narrow purpose.
7. Any general comments about the method?	In general this method would be ideal for labs that are already doing free amino acid testing using post-column derivatization HPLC.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	Yes, after limitations are discussed and evaluated.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-25 13:07:52
Name	Nour Eddine ES-SAFI
E-mail	nouressafi@yahoo.fr
Organization	Mohammed V University in Rabat
Title of Method	Analysis of Theanine in Tea (Camellia sinensis) Dietary Ingredients and Supplements by HighPerformance Liquid Chromatography with Post-Column Derivatization: Single Laboratory Validation
AOAC Candidate Method Number (e.g. ALN-01)	TEA-01
Applicable SMPR	2015.014
Summary:	The Tea-1 method presents experimental technique for Theanine analysis in dietary ingredients and supplements using simple buffer extraction followed by cation-exchange chromatography, post-column reaction with Ninhydrin reagent and UV/Vis detection
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	The AOAC SMPR 2015-014 concerns the quantitative determination of catechins, methyl xanthenes, theaflavins, and theanine. In the proposed Tea-1 method only theanine is determined.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	The analytical technique used in the Tea-1 method meet the SMPR. From all the given results only one does not meet the SMPR. (Recovery: 108.7 % instead of 107 %)
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	Accuracy and Recovery were explored using Standard Reference Materials SRM3254, SRM3255 and SRM3256.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	Yes
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	Yes. Not for all analytes in the SMPR applicability statement. Only theanine compound was investigated.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Yes. The proposed method contains injections of blank reagent, working calibration solutions and control samples.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	Yes
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes
5. Based on the supporting information, what are the pros/strengths of the method?	The Proposed method uses a cation-exchange HPLC separation technique coupled with post-column ninhydrin derivatization. This has been considered as a reference method in term of its robust nature, its quantitative performance and reproducibility. Sample components are separated in the column before the reaction, it is not affected by the sample matrix during reaction with the derivatizing reagent.
6. Based on the supporting information, what are the cons/weaknesses of the method?	Sensitivity may be questionable Relatively high reagent consumption The use of UV-visible detection The purity of the separated peaks should be checked Only one analyte (theanine) is explored
7. Any general comments about the method?	No
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	The proposed method meets the major SMPR criteria and then could be adopted (after discussion with the ERP members) as a First Action

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-08-01 15:32:08
Name	tom phillips
E-mail	tom.phillips@maryland.gov
Organization	SCS MDA
Title of Method	Analysis of Theanine in Tea (Camellia sinensis) Dietary Ingredients and Supplements by High- Performance Liquid Chromatography with Post-Column Derivatization: Single Laboratory Validation
AOAC Candidate Method Number (e.g. ALN-01)	TEA-01
Applicable SMPR	2015.014
Summary:	Theanine is extracted from the samples with Lithium Citrate buffer pH 2.2 using ultrasonic water bath. LNorleucine is used as Internal Standard. The extract is filtered and injected on a Lithium cation-exchange HPLC column and Theanine is separated from other free amino acids using Lithium citrate buffers with different pH and concentrations as mobile phases. All amino acids, including L-Theanine, react with Ninhydrin reagent in the post-column derivatization system at 130 oC and are converted to a colored derivative. Detection is performed at 570 nm using a UV/Vis detector.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	No, it does not cover the catechins, methyl xanthines, and theoflavines.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	unknown, the only analyte covered in the study is theanine.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	No, see #2
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	No, see #2 in previous section
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	No, SRM 3257 was not used.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	Only in reference to theanine.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	No, only theanine.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	Yes

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	No, only for theanine.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	No, only for theanine.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes
5. Based on the supporting information, what are the pros/strengths of the method?	The use of a post column unit for the selectivity.
6. Based on the supporting information, what are the cons/weaknesses of the method?	Only analyzed one component of tea. it is unknow if it would work for others.
7. Any general comments about the method?	Good method for theanine.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	No, it does not meet the SMPR.

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AOAC Stakeholder Panel on Dietary Supplements Expert Review Panel

AOAC Candidate Method #TEA-02

Determination of Catechins and Caffeine in *Camillia sinensis* Raw Materials, Extracts, and Dietary Supplements by HPLC-UV: Single-Laboratory Validation

- Author(s): Mark Roman, Jana Hildreth, Silvia Bannister
- Submitted by: Jana Hildreth
- Enclosures: 0
- Submitter notes: None

Link to Method: http://griegler-aoac-org.cld.bz/TEA-02

Password: a3oc

Primary Reviewer: Aniko Solyom

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-27 15:55:23
Name	Nour Eddine ES-SAFI
E-mail	nouressafi@yahoo.fr
Organization	Mohammed V University in Rabat
Title of Method	Determination of Catechins and Caffeine in Camillia sinensis Raw Materials, Extracts, and Dietary Supplements by HPLC-UV: Single-Laboratory Validation
AOAC Candidate Method Number (e.g. ALN-01)	TEA-01-02
Applicable SMPR	2015.014
Summary:	The method Tea-2 presents results dealing with the quantification of 7 catechins (C, GC, EC, EGC, GCG, ECG, EGCG) and caffein in green tea raw material, powdered extract and dietary supplements containing green tee extract. Quantitative analysis was performed by RP HPLC separation and UV detection.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes. The investigated analytes are all indicated in the applicability of the AOAC SMPR 2015.014. In addition to caffein 7 catechins are concerned by the study and included (+)-catechin, galloxcatechin, (-)-epicatechin, epigallocatechin, gallocatechin gallate, epicatechin gallate and epigallocatechin gallate
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	LOQ: not clearly indicated. LOD: not indicated. Reproducibility: not conducted. Recovery: Bad results were obtained in some cases. Repeatability: Good results were obtained with EGC, caffein, EGCG. Bad results were obtained with GC, C, EC, GCG and ECG.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	Yes

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	Standard reference material SRM 3254, SRM 3255 and SRM 3256 have been used. Information regarding repeatability (SRM 3254 and SRM 3255) with many bad results are reported.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	Yes
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	Not for all analytes in the SMPR applicability statement. methy xanthenes, theaflavins, and theanine are not included in the study.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Yes the method contains system suitability test including duplicate injections of standard solution and calibration standard.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	Repeatability results obtained using the SRM 3254 reference material showed many bad data. This is the case of GC (14.9 %), C (10.8 %), EC (5.16 %), GCG (5.72 %) and ECG (5.66 %). The AOAC SMPR 2015.014 indicated teh obtained values should be less or equal to 5. Repeatability results with the SRM 3255 reference material showed good results. Repeatability results with the SRM 3256 reference material showed good results except for GC and C where bad values have been obtained (5.57 and 8.94 % respectively).
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes
5. Based on the supporting information, what are the pros/strengths of the method?	The proposed method is rapid, simple and robust. The optimized used chromatographic conditions gave chromatographic profiles where the investigated analytes are separated during a relatively short time.
6. Based on the supporting information, what are the cons/weaknesses of the method?	Not all the method performance requirements are performed. Does not meet the SMPR
7. Any general comments about the method?	No
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	I do not recommend this method be adopted as a first action in the present form. I think that more experiments are needed and should concern all the performance requirements indicated in the 2015.014 SMPR.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-28 12:47:46
Name	Martha Jennens
E-mail	martha.jennens@covance.com
Organization	Covance
Title of Method	Determination of Catechins and Caffeine in Camillia sinensis Raw Materials, Extracts and Dietary Supplements by HPLC-UV: Single Laboratory Validation
AOAC Candidate Method Number (e.g. ALN-01)	TEA-02
Applicable SMPR	2015.014
Summary:	A rapid method to quantify seven catechins and caffeine in green tea raw material and powdered extract and dietary supplements containing green tea extract. The method utilizes RP HPLC with a phenyl column and gradient elution with UV detection and a 13 minute run time.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	No. The method only applies to seven of eight catechins, one of three methyl xanthines (caffeine) and no theaflavin or theanine is covered.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Yes, but it does not cover all analytes in the SMPR.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	There is no discussion in the method around safety/precautions in use of chemicals, etc.

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes. A single lab validation was done. Not all of the analytes in the SMPR were covered.
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	In the single lab validation three of the four reference materials were used. The supporting data demonstrates selectivity, repeatability, ruggedness and linearity. LOD and LOQ are mentioned, but no supporting data is provided. A few of the repeatability results were out of the specified range of the SMPR.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	Yes, the data meets SMPR method performance requirements for three of four reference materials were used. No other matrices were used as outlined in the SMPR.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	This method covers six of the seven catechins outlined in the SMPR. All of the others might be able to be detected with the exception of Theanine. Work would need to be done to show that they could be added to the analysis successfully.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	A statement about chemical safety needs to be added at a minimum.

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Yes, system suitability is covered.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	Yes, there is information supporting system suitability has been met per SMPR.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Method is clearly written. Would like to see data around LOQ/LOD. Also, there is a comment that centrifugation is used instead of filtration to prevent loss/binding of catechins to filters. Would like to see data in support of this.
5. Based on the supporting information, what are the pros/strengths of the method?	The method provides a rapid way to quantify seven catechins and caffeine in green tea extracts, supplements, etc. The accuracy, linearity and selectivity is good.
6. Based on the supporting information, what are the cons/weaknesses of the method?	The only weakness of this method is that it does not cover all of the analytes outlined in the SMPR.
7. Any general comments about the method?	Would like to see additional work to determine if the missing analytes could be added.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	Yes, if partnered with another method (TEA-01) for a more complete analysis of SMPR requirements.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-27 17:37:34
Name	Phil Koerner
E-mail	philk@phenomenex.com
Organization	Phenomenex
Title of Method	Determination of Catechins and Caffeine in Camillia sinensis Raw Materials, Extracts, and Dietary Supplements by HPLC-UV: Single-Laboratory Validation
AOAC Candidate Method Number (e.g. ALN-01)	TEA-02
Applicable SMPR	2015.014
Summary:	Determination of Catechins and Caffeine in Green Tea (Raw Materials, Extracts, and Dietary Supplements) by HPLC-UV on a phenyl column under gradient conditions with total run time of 13 minutes.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	yes; all 7 catechins included, plus caffeine (2 other methyl xanthines (theobromine and theophylline) not included).
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	yes

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	yes
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	yes; SRM 3254, 3255, and 3256 used
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	yes
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	No but OK since method is only applicable for catechins, but only caffeine is included from methyl xanthenes
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	no

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	yes
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	yes
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	yes
5. Based on the supporting information, what are the pros/strengths of the method?	1. appears to be a simple straight forward HPLC gradient separation with UV detection 2. baseline resolution for all analytes 3. appropriate system suitability included 4. addressing the potential for analyte degradation by inclusion of EDTA in mobile phase and diluent
6. Based on the supporting information, what are the cons/weaknesses of the method?	1. only includes caffeine of the 3 methyl xanthenes; could it be extended to include theobromine and thophylline?
7. Any general comments about the method?	method specifies Ascentis Phenyl 3um 100x3.0mm column; would other Phenyl columns also work to give equivalent results? This would give users some flexibility in column selection
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	yes method applicable to catechins and caffeine per SMPR for Tea

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-29 17:50:23
Name	Melissa Phillips
E-mail	melissa.phillips@nist.gov
Organization	NIST
Title of Method	Determination of Catechins and Caffeine in Camillia sinensis Raw Materials, Extracts, and Dietary Supplements by HPLC-UV: Single Laboratory Validation
AOAC Candidate Method Number (e.g. ALN-01)	TEA-02
Applicable SMPR	2015.014
Summary:	This method is presented for determination of seven catechins and caffeine in green tea containing dietary ingredients and supplements. The method utilizes simple sonication extraction in acidic solution containing EDTA and reversed-phase chromatography with absorbance detection. Tested products include powders and tablets. Method did not address softgels, gelcaps, gummies, chewables, or liquids.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	The method addresses the determination of seven of the eight catechins listed in the SMPR (all but catechin gallate), as well as caffeine. The method also does not address the determination of additional methyl xanthines (theophylline and theobromine), theaflavins, or theanine.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Analytical range stated in SMPR: 10-500,000 ppm. The calibration range described in method was 1-100 ppm. Lowest level tested in any product 1260 ppm (catechin in SRM 3254). Sample preparation steps need to be modified to cover the range of samples concentrations specified in the SMPR. LOQ stated in SMPR: <5 ppm. LOQ not reported in method. Recovery was tested by spiking different quantities of SRM 3255 Green Tea Extract onto a blank multivitamin matrix. Recovery was not tested at levels below 390 ppm. Recoveries were out of spec for 7 of 8 compounds at low and middle spike levels and for 3 of 8 compounds at high spike levels. 95-105% for 501-500,000 ppm RSDr was not reported below 1000 ppm. stated in SMPR was met for all conditions. RSDr was met for only 3 of 8 compounds and for total catechins. <5% for 51-500,000 ppm Tested products include powders and tablets. Method did not address softgels, gelcaps, gummies, chewables, or liquids.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	No safety information included. Might be necessary for working with concentrated acids in diluent preparation.

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes.
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	No. 3 NIST SRMs were used for demonstration of accuracy. Values reported in the method were compared to the values in the COAs from NIST. Values for GC and GCG were outside of the certified range (high) for all 3 materials. The values for EGCG in the green tea leaves and for ECG in the green tea extract were also high. This indicates a likely interference in the chromatography with GC and GCG, and possibly with the other components as well.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	No. 3 NIST SRMs were used for demonstration of accuracy. Values reported in the method were compared to the values in the COAs from NIST. Values for GC and GCG were outside of the certified range (high) for all 3 materials. The values for EGCG in the green tea leaves and for ECG in the green tea extract were also high. This indicates a likely interference in the chromatography with GC and GCG, and possibly with the other components as well.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	The method only addresses the determination of 7 catechins and caffeine. The method does not address the determination of CG, other methyl xanthines, theanine, or theaflavins. The method could potentially be extrapolated to include some of the other compounds (CG, theobromine, theophylline).
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Yes. A blank check sample could be added to comply with SMPR.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	Yes.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes. I would add more guidance under the section for sample preparation to assist in choice of sample size and extraction volume. Something like "Choose sample size corresponding to approximately X mg of catechins/analytes".
5. Based on the supporting information, what are the pros/strengths of the method?	The method is simple and straightforward (sonication extraction, chromatography, detection). This method could be easily implemented in any laboratory. The chromatography is fast and appears to separate the 8 compounds of interest.
6. Based on the supporting information, what are the cons/weaknesses of the method?	The method has not demonstrated accuracy via use of reference materials or spike/recovery studies. The method may benefit from inclusion of an internal standard. The method has not demonstrated adequate precision as specified in the SMPR. The method has not demonstrated adequate linearity, LOQ, and LOD as stated in the SMPR. Unknown how method would respond to additional matrices specified in the SMPR, particularly gummies/chewables (2 difficult matrices). Sonication extraction might not be sufficient.
7. Any general comments about the method?	This is a good start, but even for the limited number of compounds and matrices included, most of the SMPR requirements are not met. A significant amount of work is needed before this method will meet the SMPR.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	At this time, I do not recommend this method for first action status. Additional work is needed to include more matrices (and possibly more analytes), and to improve the separation and/or extraction to address the high recovery values. Inclusion of an internal standard may help. If someone is willing to take this on, the method seems to be a good place to start.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-13 08:30:13
Name	Klaus Reif
E-mail	klaus.reif@phytolab.de
Organization	PhytoLab GmbH & Co.KG
Title of Method	Analysis of Theanine in Tea (Camellia sinensis) Dietary Ingredients and Supplements by HPLC with Post-Column Derivatization I
AOAC Candidate Method Number (e.g. ALN-01)	TEA-2
Applicable SMPR	SMPR 2015.014
Summary:	The method can be used for the Determination of Theanin in Green Tea, Extracts and different Dietary Supplements using IEX-Chromatography and post-column derivatization.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	YES
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	YES
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	YES
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	YES

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	YES
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	YES
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	YES
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	YES
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	NO

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	no; Can't find a blank injection of the extraction solvent.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	YEYS
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	YES
5. Based on the supporting information, what are the pros/strengths of the method?	fully automated derivatization, as an online post-column derivatization System is used. The method is also applicable to complex dietary supplements like soft-gel capsules containing other plant extracts and a variety of excipients
6. Based on the supporting information, what are the cons/weaknesses of the method?	con: from a practical point-of-view: I would assume that only a few labs will have and use this derivatization system
7. Any general comments about the method?	I think an alternative procedure for off-line derivatization (e.g. with AQC-reagent) should be described.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	By voting strictly according to the given SMPR I have to recommend this method, as all requirements are fulfilled with excellent Validation data. From a practical point of view my concerns are that only a few labs may have this online derivatization System from Pickering. I would recommend to include an alternative for this labs. This could be an off-line derivatization e.g. with AQC-reagent, which is quick and fast and stable.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-07-30 04:41:22
Name	Darryl Sullivan
E-mail	darryl.sullivan@covance.com
Organization	Covance Laboratories
Title of Method	Determination of Catechins and Caffeine in Camillia sinensis Raw Materials, Extracts, and Dietary Supplements by HPLC-UV: Single Laboratory Validation.
AOAC Candidate Method Number (e.g. ALN-01)	TEA-02
Applicable SMPR	2015.014
Summary:	This method can quantify seven catechins and and caffeine in green tea raw materials, and powdered extracts and dietary supplements containing green tea extract. The method utilizes reverse phase HPLC with a phenyl-based stationary phase and and gradient elution. Detection is accomplished using UV absorption.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	This method is capable of measuring catechins and caffeine, but has not been demonstrated to be capable of determining methyl xanthines, theaflavins, or theanine.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	The method appears to meet the SMPR for catechins.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	yes
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	yes

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	yes
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	yes - this method used several of the NIST reference materials specified in the SMPR
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	yes
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	yes
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	no

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	yes
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	yes
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes- the method is written very clearly.
5. Based on the supporting information, what are the pros/strengths of the method?	This method is well written and easy to follow.
6. Based on the supporting information, what are the cons/weaknesses of the method?	This method has not been shown to be capable of measuring methyl xanthines, theaflavines, or theanine.
7. Any general comments about the method?	no
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	I recommend that this method be considered of AOAC Official First Action status of catechins only.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-08-01 07:57:25
Name	Kurt Young
E-mail	kurt.young@att.net
Organization	GNC/Nutra Manufacturing
Title of Method	Determination of Catechins, Methyl Xanthines in Tea
AOAC Candidate Method Number (e.g. ALN-01)	TEA-02
Applicable SMPR	2015-014
Summary:	This method determines the content of catechins and the methyl xanthine, caffeine, in tea (Camillia sinensis) raw materials, extracts and dietary supplement products using HPLC with a phenyl column and UV detection. The method is presented as the published JAOAC paper.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	Yes.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Yes.
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	Yes, although the method outlined in the journal reference appears to have been written before the SMPR was adopted.
4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	No. In that this is a journal publication, the precautions are not stated in that they represent general precautions needed for reverse phase HPLC and for the typical solvents and reagents used.

1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes.
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	Yes. All three of the available NIST SRM's were utilized.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	Yes.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	Yes.
1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	No.

2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Yes.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	Yes.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes.
5. Based on the supporting information, what are the pros/strengths of the method?	This method utilizes readily available instrumentation and reagents and gives accurate and reasonably precise results. The HPLC conditions were optimized to give relatively rapid results and the sample preparation steps were simple and straight forward. The method therefore, should be useful for quality testing purposes.
6. Based on the supporting information, what are the cons/weaknesses of the method?	The chromatography showed peak tailing and lack of baseline resolution for some of the analyte peaks. Some of the %RSD precision results were high for minor catechins. Use of all of the individual catechin standards
7. Any general comments about the method?	This method could be optimized by individual laboratories with minor modifications of chromatographic conditions. Determining relative response factors for the minor catechins might be advisable for routine quality testing work.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	Yes. The method is appropriate and applicable to routine testing for quality purposes. Additional work may need to be performed to determine the application in supplement forms not addressed in the journal reference: e.g., liquids, soft-gelatin capsules.

AOAC SPDS ERP - Set 3 Review Form 2

Submission Date	2016-08-01 16:06:04
Name	tom phillips
E-mail	tom.phillips@maryland.gov
Organization	SCS MDA
Title of Method	Determination of Catechins and Caffeine in Camillia sinensis Raw Materials, Extracts, and Dietary Supplements by HPLC-UV: Single-Laboratory Validation
AOAC Candidate Method Number (e.g. ALN-01)	TEA-02
Applicable SMPR	2015.014
Summary:	A rapid method has been developed to quantify seven catechins and caffeine in green tea (Camillia sinensis) raw material and powdered extract, and dietary supplements containing green tea extract. The method utilizes RP HPLC with a phenyl-based stationary phase and gradient elution. Detection is by UV absorbance. The total run time, including column re-equilibration, is 13 min. Single-laboratory validation (SLV) has been performed on the method to determine the repeatability, accuracy, selectivity, LOD, LOQ, ruggedness, and linearity for (+)-catechin, (-)-epicatechin, (-)-epicatechin gallate, (-)-epigallocatechin, (-)-gallocatechin gallate, (-)-epigallocatechin gallate, and (+)-gallocatechin, as well as caffeine. Repeatability precision and recovery results met AOAC guidelines for SLV studies for all catechins and caffeine down to a level of approximately 20 mg/g. Finished products containing high concentrations of minerals require the use of EDTA to prevent decomposition of the catechins.
1. Does the applicability of the method support the applicability of the SMPR? If not, please explain what is missing.	No, it does not cover the methyl xanthines((other than caffeine), theaflavins nor theanine.
2. Does the analytical technique(s) used in the method meet the SMPR? If not, please specify how it differs from what is stated in the SMPR.	Yes
3. Are the definitions specified in the SMPR used and applied appropriately in the method? If no, please indicate how the terms are used.	no, theaflavins and theanine are not used.

4. Does the method, as written, contain all appropriate precautions and warnings related to the method's reagents, components, instrumentation, or method steps that may be hazardous? If no, please suggest wording or option(s).	No safety statement present.
1. Are the definitions specified in the SMPR used and applied appropriately in the supporting documentation (manuscripts, method studies, etc...)? If not, please explain the differences and if the method is impacted by the difference.	Yes, except for theanine and theaflavins.
2. Is there information demonstrating that the method meets the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of performance of the method.	no, only 3254 and 3256 were used.
3. Is there information demonstrating that the method performs within the SMPR Method Performance Requirements using the Reference Materials stated in the SMPR? If not, then specify what is missing and how this impacts demonstration of method performance.	Yes for the compounds studied. Not all SMR's were used.
4. Is there information demonstrating that the method performs within the SMPR Method Performance REquirements table specifications for all analytes in the SMPR applicability statement? If not, please specify what is missing and whether or not the method's applicability should be modified.	Yes for the compounds studied. Not all SMR's were used.

1. Based on the supporting information, were there any additional steps in the evaluation of the method that indicated the need for any additional precautionary statements in the method?	no safety statement present
2. Does the method contain system suitability tests or controls as specified by the SMPR? If not, please indicate if there is a need for such tests or controls and which ones.	Yes for the compounds studied.
3. Is there information demonstrating that the method system suitability tests and controls as specified in the SMPR worked appropriately and as expected? If no, please specify.	Yes for the compounds studied.
4. Based on the supporting information, is the method written clearly and concisely? If no, please specify the needed revisions.	Yes
5. Based on the supporting information, what are the pros/strengths of the method?	short run time, it should easily convert to UPLC, baseline resolves the compounds studied.
6. Based on the supporting information, what are the cons/weaknesses of the method?	it did not address the theaflavins, theobromine nor theophylline.
7. Any general comments about the method?	The method is good for the compounds studied.
Do you recommend this method be adopted as a First Action and published in the Official Methods of Analysis of AOAC INTERNATIONAL? Please specify rationale.	No it does not meet the SMPR.

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

AOAC Stakeholder Panel Voting Members

AOAC INTERNATIONAL (AOAC) assembles stakeholder panels to develop voluntary consensus standards. While AOAC maintains transparency and openness in accordance with national and international guidance and regulations for standards development and its policies and procedures for assembling stakeholder panels, its policies and procedures also ensures that there is a balance of interests and perspectives in achieving consensus of the stakeholder panel.

Due Process and Balance

All AOAC stakeholder panels are diverse and can vary in size. Where a stakeholder panel is not balanced or if it is significantly large whereby consensus of the general assembly may be impractical, a balanced representative voting panel will be used to demonstrate consensus. AOAC encourages ALL stakeholders to participate in deliberations during stakeholder panel meetings and working group meetings, in addition to participating during any posted comment periods. To ensure that there is a balance of interests and perspectives, a ***representative subset*** of the stakeholder panel, the voting members, is selected to reach consensus for the development of AOAC voluntary consensus standards.

Composition

Voting members represent the perspectives of the larger stakeholder panel. The voting members consist of no more than ¼ to 1/3 of the total number of stakeholders in registered. Primary and secondary representative voting members are approved. Every attempt is made to approve a panel of voting members that represents all perspectives of the stakeholder panel. In the event of a primary voting member is not able to attend, and no alternate has been approved, the stakeholder panel chair, working with AOAC can provisionally approve an alternate from those in attendance to assure balance and lack of dominance. For stakeholder panels with scopes including diverse topics, the voting member representatives may be rotated to include other stakeholders for successive meetings to ensure a lack of dominance by any particular stakeholder.

Approval Process

AOAC works with the chair of the stakeholder panel and potentially other key stakeholders to develop a proposed representative voting member panel. Following AOAC policies and procedures, the proposed voting members and documentation are submitted to the AOAC Official Methods Board (OMB) for review and approval. The OMB's review ensures that the proposed panel is balanced in interests and perspectives representing the stakeholder panel and a lack of dominance.

Roles and Responsibilities

Every stakeholder has a voice and every stakeholder is entitled to state his/her or organizational perspective(s). This is due process. In developing AOAC standards, stakeholder consensus is demonstrated by 2/3 vote (67%) in favor of a motion to adopt a standard. It is important to note: Individual voting members do not have any additional weight, voice or status in stakeholder deliberations than other stakeholders. The role of the voting members is to demonstrate the consensus of the stakeholder panel. Voting members may vote in favor or against any motion and/or they may abstain. Stakeholder panel chair will moderate voting process. AOAC carefully documents the vote. It is important for voting members to be in the room during the time for voting. It is also important for voting members to inform the chair of his/her inability to serve as a voting member.

AOAC INTERNATIONAL

STAKEHOLDER PANEL ON DIETARY SUPPLEMENTS (SPDS)
WORKING GROUP CHAIR & MEMBERS

VOLUNTEER ROLE DESCRIPTION

POSITION TITLE: **Working Group Chair and Members, AOAC SPDS Working Group**
POSITION CLASSIFICATION: **Volunteer**
REPORTS TO: **SPDS Chair**
DATE PREPARED: **March 13, 2014**

POSITION SUMMARY:
In keeping with the mission of AOAC INTERNATIONAL and the goals of the Stakeholder Panel on Dietary Supplements (SPDS), working group chairs will lead their working group in the development of standards (or other tasks as assigned by the SPDS chair) for specific priority ingredients as defined by the SPDS Advisory Panel. Working group chair(s) will work with AOAC staff and stakeholders to meet the working group's goals and disseminate recommendations to the stakeholder panel and community at-large. The working group may hold meetings in person and/or via teleconference (web and video) to complete its work. The chair of the working group will moderate the working group discussions, assist in scheduling the meetings, and report the working group's recommendation back to SPDS. Working group chairs will work with AOAC to formulate the working group's recommendations into motions for SPDS's consideration.

ELIGIBILITY CRITERIA FOR SPDS WORKING GROUP CHAIR:
- Must be a key expert and/or thought leader in dietary supplements and the technologies used for priority ingredients as assigned for the specific working group.
- Must have the recommendation of the SPDS Chair.

WORKING GROUP CHAIR RESPONSIBILITIES:
- Chair meetings of the working group, moderate discussions of the working group and work with AOAC staff to facilitate working group's work.
- Work with AOAC staff and SPDS chair to identify working group members, any additional expertise/resources needed facilitate the work of the working group.
- Work as a team member and also independently.
- Present an overview on the specified priority ingredient under consideration including, but not limited to, regulatory implications, and public health and public safety challenges with methodology.
- Prepare a draft fitness for purpose statement for specified priority ingredient and technology to present to SPDS for consideration.
- Work with AOAC staff to reconcile actions and outcomes of working group deliberations.
- Using AOAC guidance to reconcile comments and address questions on SMPR.
- Present working group recommended SMPR to SPDS for review and approval.
- Work with AOAC staff and stakeholders to draft and review relevant methodology and working group documentation.
- Draft SMPR white paper for publication.
- Perform duties and reviews in timely fashion.
- Other tasks as agreed upon by working group chair, SPDS chair and AOAC staff.

DUTIES AND RESPONSIBILITIES OF THE SPDS WORKING GROUP MEMBERS:
The working group will meet either in person and via teleconference, web conferencing or by other means of communication. All communication and meetings of the working group must be facilitated through AOAC

staff. The working group's tasks will include developing standard method performance requirements (SMPRs), review of methodology, identifying expertise and other as may be requested by the SPDS chair. Working groups are not required to vote, but to show general consensus for its recommendations. The groups should meet to discuss their objectives and complete their assigned tasks. Individuals on the working groups may be tasked with their own action items and responsibilities. More than one meeting and one round of communication may be required to complete the working group's tasks. All working group participants are expected to contribute and are expected to have completed the SMPR Education Session. AOAC staff will document all working group decisions and actions.

AOAC RESOURCES:

- Referencing AOAC guidance documentation to assist in drafting the fitness for purpose statement, standard method performance requirements (SMPR), and additional work as tasked.
    1) AOAC Fitness for Purpose Statement Guideline
    2) Appendix F: Guidelines for Standard Method Performance Requirements
    3) Appendix K: Guidelines for Dietary Supplements and Botanicals

STAFF LIASON:

AOAC will assign staff to facilitate the work of the working group.

TERMS OF REVIEW:

This document will be reviewed biannually by the SPDS Chair and AOAC staff.

DATES REVISED:

Stakeholder Panel

Voting Panel – A vetted, representative, and balanced subset of the assembled stakeholders. Ideally the number of voters represents $^1/_4$ to $^1/_3$ of the assembly.

Voting Guidelines – A. motions to create a consensus based standard (ex: voting on fitness for purpose statements or Standard Method Performance Requirements) require a 2/3 vote for the motion to carry.
B. Any other motion (ex: votes to clarify information for working groups, set priorities or direction, etc.) requires a majority vote to carry.

Expert Review Panel

Voting Panel – 7 – 10 vetted experts
Quorum - The presence of **7** members or **2/3** of total vetted ERP membership, whichever is greater.
Voting Guidelines – Motions to adopt a *First Action Official Method℠ of Analysis* carry by unanimous vote on first ballot. If not unanimous, negative votes must delineate scientific reasons, and can be overridden by 2/3 of voting ERP members after due consideration. Dissenting opinions are recorded.

Working Group

Voting Panel – There is no formal voting panel. Any interested and knowledgeable party may participate. Working groups sole purpose is to provide recommendations to stakeholder panels.

Voting Guidelines –majority vote carries all motions, dissenting opinions considered by assembly and recorded.

Helpful Definitions & Terminology

Quorum	The number of members who must be present in order to validly transact business. It is determined by the number of members present, not the number present and voting. (*Fundamentals of Parliamentary Law and Procedure, 3rd edition. p. 151*).
Representative Voting Panel Members	Every member has an obligation to vote and the right to abstain.
Abstentions	Abstentions reduce the number required to obtain a majority of those present and voting. They are only counted to confirm the presence of a quorum. (*Fundamentals of Parliamentary Law and Procedure, 3rd edition. p. 237).*
Order	Meetings should address only one item of business at one time (only one pending motion at a time). Chairs should not permit digression or introduction of different topics until the business at hand is resolved. No pending motions while changing topics. (*Fundamentals of Parliamentary Law and Procedure, 3rd edition. p. 1).* All business must be conducted with order and should be done fairly and impartially. The presiding officer should impartially ensure that each member has an opportunity to speak. *(Fundamentals of Parliamentary Law and Procedure, 3rd edition. pp. 1-2).*
Equality	All members have equal opportunity to propose motions, to participate in debate, to vote, to serve on committees or as an officer, to share in activities according to the member's abilities. *(Fundamentals of Parliamentary Law and Procedure, 3rd edition. p. 2).*
Justice	All members have the right to ask questions, to be informed, to have complex motions explained by the chair. *(Fundamentals of Parliamentary Law and Procedure, 3rd edition. p. 2).*
Minority Rights	Dissenting members have equal rights to voice opposing or minority opinions and strive to become the majority. *(Fundamentals of Parliamentary Law and Procedure, 3rd edition. p. 2).*
Majority Rights	No members, board, or officers have the right to dictate or control decisions unless the member grant such rights Members may not take any action in conflict with federal, regional or organizational laws or policies. Decisions are based on the will of the majority. *(Fundamentals of Parliamentary Law and Procedure, 3rd edition. p. 2).*

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.

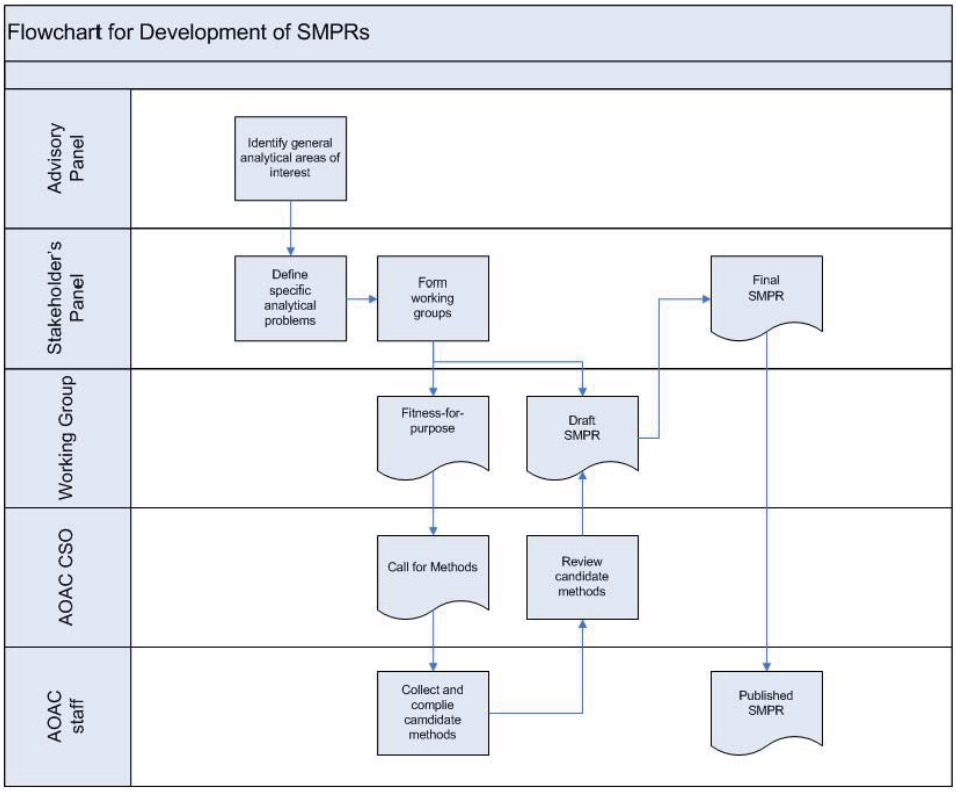


Figure 1. Schematic flowchart diagram of the SMPR development process.

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.

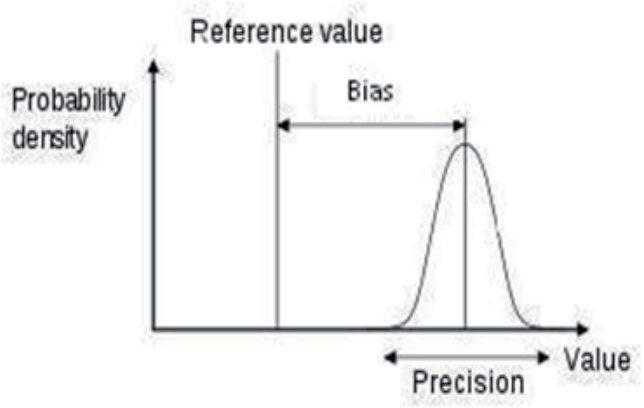


Figure A1. Relationship between precision versus bias (trueness). Trueness is reported as bias. Bias is defined as the difference between the test results and an accepted reference value.

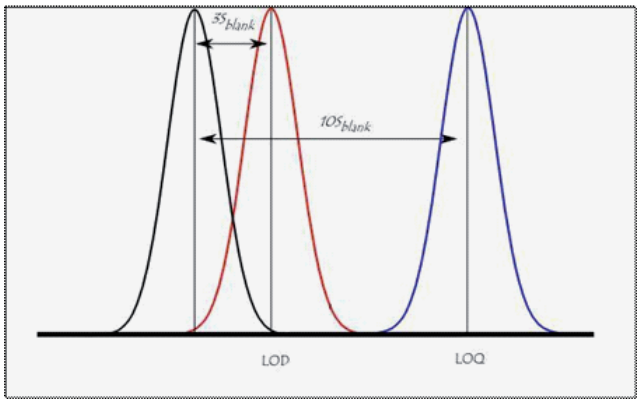


Figure A2. Relationship between LOD and LOQ. LOD is defined as the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit. LOQ is the level above which quantitative results may be obtained with a stated degree of confidence.

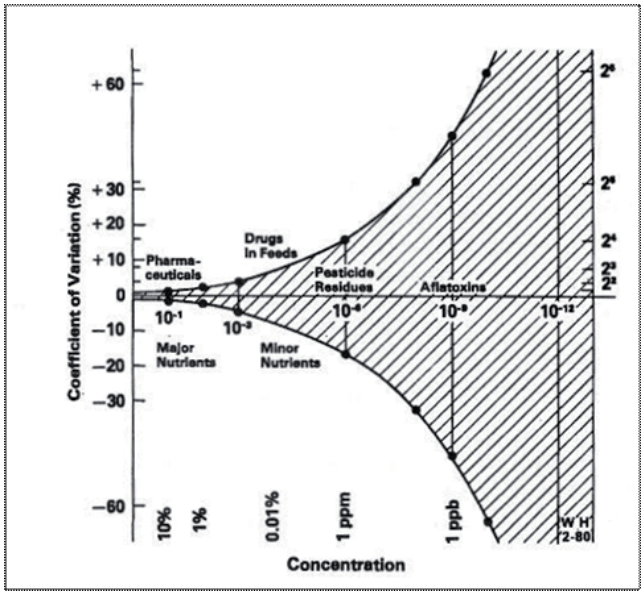


Figure A3. Horwitz Curve, illustrating the exponential increase in the coefficient of variation as the concentration of the analyte decreases [*J. AOAC Int*. 89, 1095(2006)].

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.

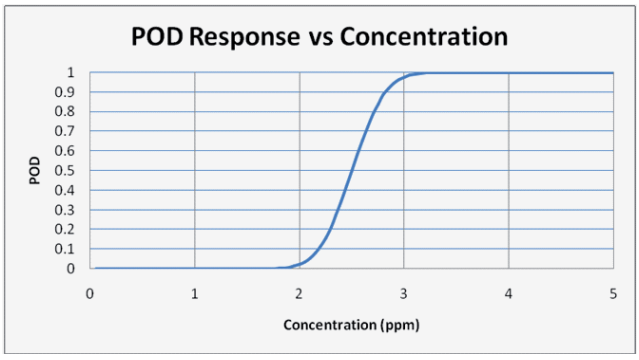


Figure C1. Theoretical POD curve for a qualitative detection method.

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

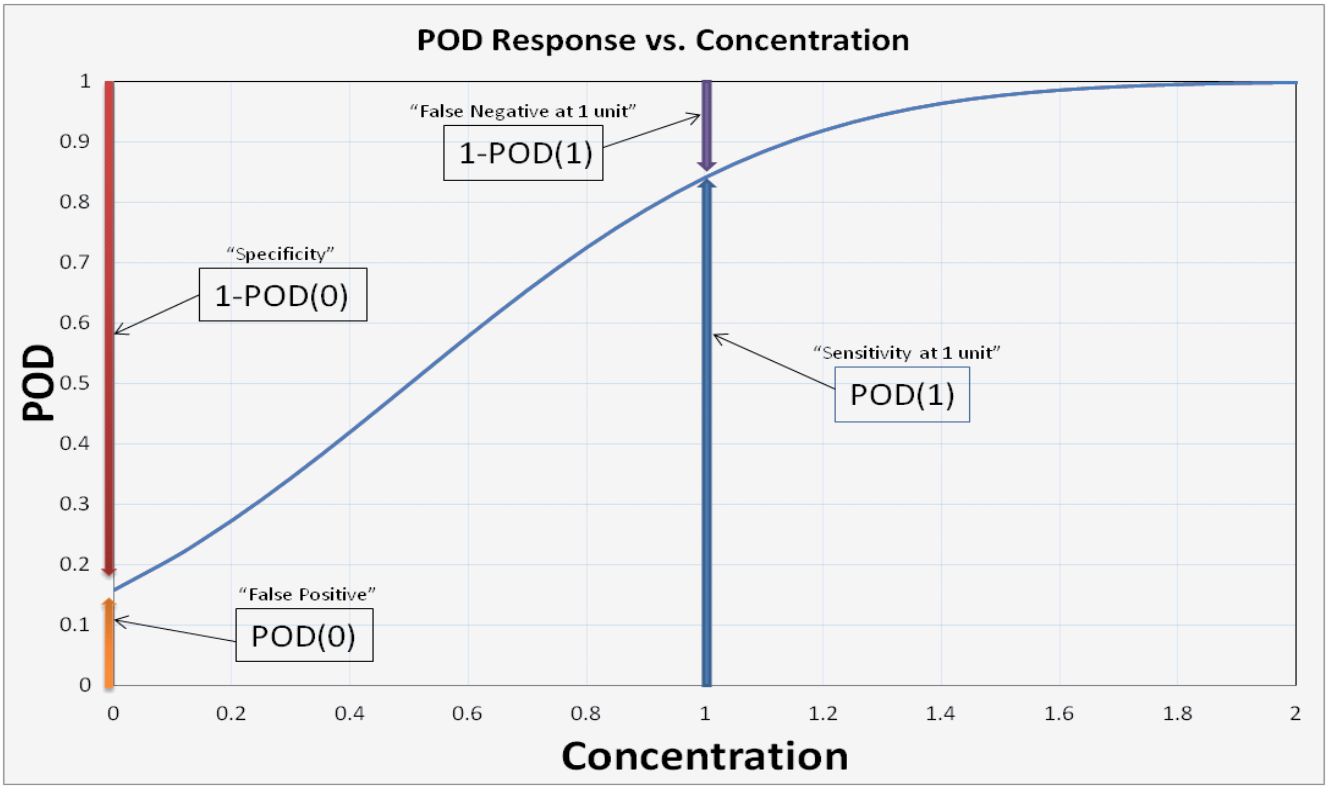


Figure C2. Comparison of POD model terminology to other obsolete terms.

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.

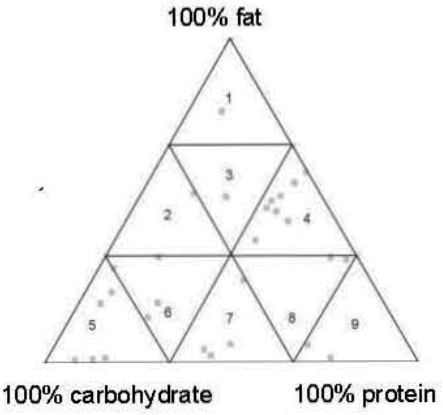
Why Use an In-House Reference Material?

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.