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SOLICITATION AMENDMENT
MODIFICATION DE L'INVITATION

The referenced document is hereby revised; unless otherwise indicated, all other terms and conditions of the Solicitation remain the same.

Ce document est par la présente révisé; sauf indication contraire, les modalités de l'invitation demeurent les mêmes.

Comments - Commentaires

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Title - Sujet CHEMISTRY TESTING	
Solicitation No. - N° de l'invitation 39903-130313/A	**Amendment No. - N° modif.** 001
Client Reference No. - N° de référence du client 39903-130313	**Date** 2013-02-21
GETS Reference No. - N° de référence de SEAG PW-$$SS-013-25446	
File No. - N° de dossier 013ss.39903-130313	**CCC No./N° CCC - FMS No./N° VME**
Solicitation Closes - L'invitation prend fin **at - à 02:00 PM** **on - le 2013-03-15**	**Time Zone** **Fuseau horaire** Eastern Standard Time EST
F.O.B. - F.A.B. **Plant-Usine:** ☐ **Destination:** ☑ **Other-Autre:** ☐	
Address Enquiries to: - Adresser toutes questions à: Dagenaig, Gaëtane	**Buyer Id - Id de l'acheteur** 013ss
Telephone No. - N° de téléphone (819) 956-1365 ( )	**FAX No. - N° de FAX** (819) 997-2229
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Name and title of person authorized to sign on behalf of Vendor/Firm (type or print) **Nom et titre de la personne autorisée à signer au nom du fournisseur/ de l'entrepreneur (taper ou écrire en caractères d'imprimerie)** **Signature** **Date**	

This Solicitation Amendment is raised to reflect revisions to Part 4, Evaluation Procedures and Basis of Selection, Part 7, Resulting Contract Clauses, Attachment 2 to Part 4, and to two (2) attachments of Appendix 1 to Annex A, Reference Methods and Criteria in the English version only:

Under Part 4, Evaluation Procedures and Basis of Selection,
At article 2.1.2, For each Survey:

Delete: "The evaluated price for each Survey will be determined on the basis of items 1 to 5 of Attachment 1 to Part 3, Financial Bid Presentation Sheet."

Insert: "The evaluated price for each Survey will be determined on the basis of items 1 to 5 and item 7. of Attachment 1 to Part 3, Financial Bid Presentation Sheet."

Under Part 4, Evaluation Procedures and Basis of Selection,
At article 2.1.4, For Additional Analytical Testing Services:

Delete: "Selection of a contractor to carry out these services on an as and when required basis will be in accordance with the ranking methodology included in article 1.5 of Part 7 - Resulting Contract Clauses."

Under Part 7, Resulting Contract Clauses,
At article 1.5 Task Authorization - Order of Ranking for Additional Analytical Testing Services:

Delete: in it entirety.

Under Attachment 2 to Part 4, Evaluation of Price,

Delete :"Total evaluated price for items 1. to 5. for each Survey for all periods:

Total for 1. for each survey + Total for 2. for each survey + Total for 3. for each survey + Total for 4. for each Survey + Total for 5. for Expert testimony"

Insert: **"**Total evaluated price for items 1. to 5. for each Survey for all periods:

Total for 1. for each survey + Total for 2. for each survey + Total for 3. for each survey + Total for 4. for each Survey + Total for 5. for Expert testimony + Total for C.1 Additional Analytical Testing services"

Under Attachment 2 to Part 4, Evaluation of Price,
Under Example A:

Delete: "Total evaluated price for items 1. to 5. for Acrylamide = $ 60,750.00 + $0.00 + $0.00 + $0.00 + $1340.00 = $62,090.00"

Insert: "Total evaluated price for items 1. to 5. and item C.1 for Acrylamide = $ 60,750.00 + $0.00 + $0.00 + $0.00 + $1340.00 + $4,000.00 = $66,090.00"

Under the French version only of Attachment 2 to Part 4, Evaluation of Price,

Delete : " C. 1. Le prix évalué pour les services d'essais analytiques supplémentaires sera calculé comme suit :

Sous-totaux: en multipliant le taux horaire ferme tout compris pour chacune des 3 périodes contractuelles et chacune des 2 périodes optionnelles, par le nombre estimé de jours dans la fiche de présentation de la soumission financière

Total au point B.1. = Somme des totaux pour les essais analytiques supplémentaires pour chacune des 3 périodes contractuelles et chacune des 2 périodes optionnelles

Prix total évalué pour le point B.1. pour les essais analytiques supplémentaires pour toutes les périodes

Total au point B.1. pour les services d'essais analytiques supplémentaires pour les 3 périodes contractuelles et les 2 périodes optionnelles
TPS / TVH en sus "

Insérer : "C. 1. Le prix évalué pour les services d'essais analytiques supplémentaires sera calculé comme suit :

Sous-totaux: en multipliant le taux horaire ferme tout compris pour chacune des 3 périodes contractuelles et chacune des 2 périodes optionnelles, par le nombre estimé de jours dans la fiche de présentation de la soumission financière

Total au point C.1. = Somme des totaux pour les essais analytiques supplémentaires pour chacune des 3 périodes contractuelles et chacune des 2 périodes optionnelles

Prix total évalué pour le point C.1. pour les essais analytiques supplémentaires pour toutes les périodes

Total au point C.1. pour les services d'essais analytiques supplémentaires pour les 3 périodes contractuelles et les 2 périodes optionnelles
TPS / TVH en sus"

Under the English version of Appendix 1 to Annex A, Reference Methods and Criteria:

Delete: Attachment 2 to Appendix 1, Speciation of Arsenic in a Variety of Foods:

Insert: Attachment 2 to Appendix 1, Speciation of Arsenic in a Variety of Foods, version 2

Under the English version of Appendix 1 to Annex A, Reference Methods and Criteria:

Delete: Attachment 6 to Appendix 1, Glycoalkaloids Analytical Method

Insert: Attachment 6 to Appendix 1, Glycoalkaloids Analytical Method, Version 2

Attachment 2 to Appendix 1, version 2

This document is CONFIDENTIAL when completed

Section and Section Code: Chemistry CHE

Standard operating Document Number: SOM-DAR-CHE-053-04 RE-WRITE

Document Title: Speciation of Arsenic in a Variety of Foods

APPROVAL COVER PAGE

This cover page is proof of final approval of this standard operating document. Upon the issuance of a new version of the document a new approval page will be included. The obsolete approval page will be archived with the master copy of the obsolete document.

	NAME	SIGNATURE	DATE
Modified By	**Melanie Casey**		
Originator	**Connie Samson**		
Laboratory Director	**William Lanterman**		
Section Manager	**Cory Murphy**		
Reviewed by Quality Committee	**Bree-Ann Lightfoot, QAO**		

Activation Date: 20121025

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SOM-CHE-053-04

SPECIATION OF ARSENIC IN A VARIETY OF FOODS

1 PURPOSE

1.1 This method provides the specific information required for the quantitative determination of inorganic arsenic (arsenic acid and arsenious acid) and organic arsenic (arsenobetaine, arsenocholine, monomethyl arsonic acid, cacodylic acid) in food by Liquid Chromatography - Inductively Coupled Mass Spectrometry (LC-ICP-MS)

2 REFERENCES

2.1 Charlebois, R. And Godefroy, S.B. 2008. 2008 Food Safety Science Committee Summary Report.

2.2 D'Amato. M., Forte, G., and Caroli, S. 2004. Identification and Quantification of Major Species of Arsenic in Rice. *J. AOAC Int.* Vol. 87 (1), 238-243.

2.3 Kohlmeyer, U., Jantzen, E., Kuballa, J., and Jakubik, S. 2003. Benefits of High Resolution IC-ICP-MS for the Routine Analysis of Inorganic Arsenic Species in Food Products of Marine and Terrestial Origin. *Anal Bioanal Chem*. Vol. 377, 6-13.

2.4 Heitkemper, D.T., Vela, N.P., Stewart, K.R., and Westphal, C.S. 2001. Determination of Total and Speciated Arsenic in Rice by Ion Chromatography and Inductively Coupled Plasma Mass Spectrometry. *J. Anal. At. Spectrom*. Vol. 16, 299-306.

2.5 Vela, N.P., and Heitkemper, D.T. 2004. Total Arsenic Determination and Speciation in Infant Food Products by Ion Chromatography-Inductively Coupled Plasma-Mass Spectrometry. *J. AOAC Int*. Vol. 87 (1), 244-252.

2.6 Almela, C., Laparra, J-M., Vélez, D., Barberá, R., Farré, R., and Montora, R. 2005. Arsenosugars in Raw and Cooked Edible Seaweed: Characterization and Bioaccessibility. *J. Agric. Food Chem*., Vol. 53 (18), 7344-7351.

2.7 Control Charts, Standard Operating Procedures SOP-DAR-LAB-002.

2.8 FDA Foods Program Guidelines for Chemical Methods. Version 1.0 2/28/2012. US Food and Drug Administration Office of Foods. Guidelines for the Validation of Chemical Methods for the FDA Foods Program.

2.9 EU Document No. SANCO/12495/2011. "Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed".

3 SCOPE

3.1 This method has been validated for the analysis of speciated arsenic in infant rice cereal, pear-based pureed baby food, and water. This method is suitable for food products that are enzymatically digestible or that can be diluted, filtered, and run without further processing (e.g. water and juice).

3.2 For analytical ranges refer to Appendix 2, Table 1.

3.3 Only trained and authorized analysts shall perform this analysis.

4 DEFINITIONS

4.1 Species - refers to different forms of a single element. Typically, references to chemical species are used to differentiate valence states of an element (e.g. As^{+3} and As^{+5}), or inorganic and organic elemental compounds (e.g. CH_3Hg^+ and Hg).

4.2 Speciation - refers to the separation, and usually quantitation, of different species of a specific element.

4.3 As III – Arsenious Acid, an inorganic arsenic species with a valence state of $^{+3}$, i.e. As^{+3}

4.4 As V- Arsenic Acid, an inorganic arsenic species with a valence state of +5, i.e. As^{+5}

4.5 Arsenic Equivalent - the concentration of Arsenic, when forming part of a molecule, in terms of elemental Arsenic (74.9 g/mole).

5 EQUIPMENT AND MATERIALS REQUIRED

5.1 Equipment

5.1.1 Teflon spatula or equivalent

5.1.2 Food processor, Cuisinart, Robotcoupe Blixer BX64 or equivalent

5.1.3 Spex 6970EFM Cryomill and associated accessories, or equivalent

5.1.4 Balance suitable for ± 0.01g and ± 0.0001g

5.1.5 Adjustable volume pipetters, various

5.1.6 Pasteur pipets or plastic transfer pipets

5.1.7 50 mL disposable plastic tubes and caps, Digitubes or equivalent. Rinse with de-ionized water and allow to dry prior to use.

5.1.8 50 mL graduated polypropylene (PP) centrifuge tubes with caps. Rinse with de-ionized water and allow to dry prior to use.

5.1.9 Micro-centrifuge tubes

5.1.10 14 mL disposable polypropylene round bottom tubes, 17 x 100 mm with snap cap, Falcon tubes or equivalent

5.1.11 Teflon bottles – various volumes

5.1.12 Adjustable volume dispenser

5.1.13 Volumetric flasks – various volumes

5.1.14 pH meter with calibrating solutions

5.1.15 Stirring plate with stir bars

5.1.16 Vortex mixer

5.1.17 Rotating shaker capable of running for an extended amount of time (minimum of 16 hours) and capable of holding a rack of 24 digestion tubes

5.1.18 General purpose incubator, VWR model 1545 or equivalent, capable of holding a rotating shaker, set at 37ºC ± 3ºC

5.1.19 Centrifuge, Beckmann Coulter Allegra X-15R or equivalent, capable of 4750 rpm (5250g)

5.1.20 Micro-centrifuge, VWR Galaxy 16D or equivalent, capable of 14000 rpm (16000g)

5.1.21 13 or 25 mm x 0.20 µm Nylon syringe filters or equivalent

5.1.22 Luer Lock syringes, 3cc

5.1.23 0.20 µm Nylon vacuum filters, Phenomenex or equivalent

5.1.24 Perkin Elmer ICP-MS, Elan DRC II, or equivalent system, capable of detecting masses in the range of 5 to 250 AMU and physically interfaced to analyze LC column effluent

5.1.25 High Performance Liquid Chromatograph (HPLC) capable of generating a reliable flow rate of 2mL/min at pressures up to 2500 psi with an auto sampler capable of injections up to 500 µL in a single injection

5.1.25.1 Hamilton PRP-X100 anion exchange LC column, 250 mm x 4.1 mm x 10 µm particle size, Part No. 79433 or equivalent

5.1.25.2 Hamilton PRP-X100 guard column, Part No. 79446 or equivalent

5.1 Reagents

5.2.1 Deionized water (DIW), ≥18.0 MΩ•cm

5.2.2 Methanol (MeOH), Caledon, distilled in glass grade, Catalogue No. 6701-2 or equivalent.

5.2.2.1 Extraction solution (25% MeOH v/v): Add 250 mL methanol to a 1 L volumetric flask. Make to volume with DIW. Invert to mix.

5.2.3 Concentrated Hydrochloric Acid (HCl), 36.5 - 38%, J.T. Baker, ACS reagent grade, Catalogue No. 9530-33 or equivalent.

5.2.3.1 5M HCl: Add 41 mL of concentrated HCl to approximately 60 mL of DIW in a 100 mL volumetric flask. Make to volume with DIW. Invert to mix.

5.2.4 Concentrated Ammonium Hydroxide (NH_4OH or aq. NH_3), 28.0 - 30%, BDH Chemicals, AnalaR grade or equivalent, Catalogue No. B10011 or equivalent aqueous ammonia.

5.2.4.1 12% NH_3 (v/v): Add 12 mL of concentrated NH_3 to approximately 60 mL of DIW in a 100 mL volumetric flask. Make to volume with DIW water. Invert to mix.

5.2.5 Pronase (Protease) from *Streptomyces griseus*, Sigma Aldrich, Catalogue No. P-5147 or equivalent Protease enzyme. Store frozen.

5.2.5.1 Protease solution (60mg/0.5mL): Dissolve 1.680g Protease in 14 mL DIW (This is sufficient for 24 tubes). Prepare daily.

5.2.5.2 Protease solution (30mg/0.5mL): Dissolve 0.840g Protease in 14 mL DIW (This is sufficient for 24 tubes). Prepare daily.

5.2.6 Lipase from porcine pancreas, MP Biomedicals, Catalogue No. 100817 or equivalent Lipase. Store frozen.

5.2.7 alpha-Amylase, Sigma-Aldrich, 5,000,000 units from porcine pancreas, Catalogue No. A-3176 or equivalent. Refrigerate.

5.2.8 Lipase / alpha-Amylase solution (30mg/1.0mL) Dissolve 0.750g Lipase and 0.750g alpha-Amylase in 25 mL 25% MeOH (This is sufficient for 24 tubes). Make daily.

5.2.9 Ammonium Carbonate $(NH_4)_2CO_3$, Fisher, HPLC grade or equivalent, Catalogue No. A651-500.

5.2.10 L-Tartaric Acid, $(CHOH)_2(CO_2H)_2$, EMD Science, ACS grade, Catalogue No. TX0010-1, or equivalent.

5.2.11 Reference Material with a certified value for total arsenic. (e.g. NIST 1568a Rice Flour or NIST 1643e Trace Elements in Water).

5.2.12 Primary Standards

5.2.12.1 1000 mg/L As^{+3} in 2% HCl, SpexCertiprep, Catalogue No. SPEC-AS3 or equivalent. Refrigerate.

5.2.12.2 1000 mg/L As^{+5} in water, SpexCertiprep, Catalogue No. SPEC-AS5 or equivalent. Refrigerate.

5.2.12.3 Arsenocholine (AsC), Wako Chemicals, Catalogue No. 328-34921 or equivalent. Store at room temperature.

5.2.12.4 Arsenobetaine (AsB), Fluka Chemicals, purum p.a; 95%, Catalogue No. 11093 or equivalent. Store at room temperature.

5.2.12.5 Disodium methyl arsonate hexahydrate (MMA), Supelco, 97.5%, Catalogue No. PS-281 or equivalent. Store at room temperature.

5.2.12.6 Cacodylic Acid (DMA, Dimethyl Arsenic Acid), Sigma Aldrich, 98%, Catalogue No. C0125-10g or equivalent. Store at room temperature.

Note: All standards expire one year from stock standards preparation date.

5.2.13 Stock Standards

5.2.13.1 AsC (2000 µg/mL) Weigh 0.0200 g of AsC into a 10mL volumetric flask. Add approximately 5 mL of 25% MeOH v/v and invert to dissolve. Make to volume with 25% MeOH v/v and invert to mix. Refrigerate.

5.2.13.2 AsB (500 µg/mL) Weigh 0.0050 g of AsB into a 10 mL volumetric flask. Add approximately 5 mL of 25% MeOH v/v

and invert to dissolve. Make to volume with 25% MeOH v/v and invert to mix. Refrigerate.

5.2.13.3 MMA (2000 µg/mL) Weigh 0.0200 g of MMA into a 10 mL volumetric flask. Add approximately 5 mL of 25% MeOH v/v and invert to dissolve. Make to volume with 25% MeOH v/v and invert to mix. Refrigerate.

5.2.13.4 DMA (1000 µg/mL) Weigh 0.0100 g of DMA into a 10 mL volumetric flask. Add approximately 5 mL of 25% MeOH v/v and invert to dissolve. Make to volume with 25% MeOH v/v and invert to mix. Refrigerate.

5.2.13.5 As^{+3} (1000 mg/L) refer to 5.2.12.1.

5.2.13.6 As^{+5} (1000 mg/L) refer to 5.1.12.2.

5.2.14 Intermediate Standards

5.2.14.1 As^{+3}/DMA Intermediate Standard Solution (As^{+3} - 20 µg/mL; DMA - 10 µg/mL): Transfer 0.5 mL of As^{+3} Stock Standard and 0.25 mL of DMA Stock Standard into a 25 mL volumetric flask. Make to volume with 25% MeOH. Refrigerate.

5.2.14.2 As^{+5}/AsC/AsB Intermediate Standard Solution (As^{+5} - 14 µg/mL; AsC – 20 µg/mL; AsB - 10 µg/mL): Transfer 0.35 mL of As^{+5} Stock Standard and 0.25 mL of AsC Stock Standard and 0.50 mL of AsB Stock Standard into a 25 mL volumetric flask. Make to volume with 25% MeOH. Refrigerate.

5.2.14.3 MMA Intermediate Standard Solution (28 µg/mL): Transfer 0.35 mL of MMA Stock Standard into a 25mL volumetric flask. Make to volume with 25% MeOH. Refrigerate.

5.2.15 Working Standards

5.2.15.1 High Level As^{+3}/DMA Working Standard (As^{+3} – 2.0 µg/mL; DMA – 1.0 µg/mL): Transfer 2.5 mL of As^{+3}/DMA Intermediate Standard to a 25 mL volumetric flask. Make to volume with 25% MeOH. Refrigerate.

5.2.15.2 Low Level As^{+3}/DMA Working Standard (As^{+3} – 200 ng/mL; DMA – 100 ng/mL): Transfer 2.5 mL of High Level As^{+3}/DMA Working Standard to a 25 mL volumetric flask. Make to volume with 25% MeOH. Refrigerate.

5.2.15.3 As^{+5}/AsC/AsB Working Standard (As^{+5} – 140 ng/mL; AsC – 200 ng/mL; AsB – 100 ng/mL): Transfer 0.25 mL of As^{+5}/AsC/AsB Intermediate Standard into a 25 mL volumetric flask. Make to volume with 25% MeOH. Refrigerate.

5.2.15.4 MMA Working Standard (694.4 ng/mL): Transfer 1.24 mL of MMA Intermediate Standard into a 50 mL volumetric flask. Make to volume with 25% MeOH. Refrigerate.

5.2.15.5 General Spiking Solution: Transfer the volume of stock standard solutions as indicated in Table 1 into a 25 mL volumetric flask. Make to volume with 25% MeOH. Refrigerate.

Table 1: Guide to preparation of General Spiking Solution

As Species	Volume of Stock Standard Solution (mL)	Concentration (µg/mL)
As^{+3}	0.25	10.0
AsC	0.03	2.40
AsB	0.08	1.60
As^{+5}	0.04	1.60
DMA	0.30	12.0
MMA	0.15	12.0

5.2.15.6 Water/Juice Spiking Solution: Transfer the volume of stock standard solutions as indicated in Table 2 into a 25 mL volumetric flask. Make to volume with 25% MeOH. Refrigerate.

Table 2: Guide to preparation of Water/Juice Spiking Solution

As Species	Volume of Stock Standard Solution (mL)	Concentration (µg/mL)
As^{+3}	0.125	5.0
AsC	0.025	2.0
AsB	0.050	1.0
As^{+5}	0.025	1.0
DMA	0.125	5.0
MMA	0.100	8.0

5.2.16 LC Mobile Phases (All Matrices other than seaweed)

5.2.16.1 10 mM Ammonium Carbonate with 2.5 mM Tartaric Acid in 2% Methanol, pH 8.7 (Mobile Phase A): Weigh 0.9606 g of ammonium carbonate and 0.3752 g of tartaric acid into a 1 L volumetric flask. Add 20 mL of methanol. Add approximately 500 mL of DIW and mix to dissolve the reagents. Once dissolved, make to volume with DIW. Invert to mix. Vacuum filter the solution through a 0.20 µm Nylon filter. Adjust to pH 8.7 with either 12% (v/v) NH_3 or 5M HCl. If possible, prepare fresh on the day of analysis.

5.2.16.2 30 mM Ammonium Carbonate with 2.5 mM Tartaric Acid in 2% Methanol, pH 8.7 (Mobile Phase B): Weigh 2.8818 g of ammonium carbonate and 0.3752 g of tartaric acid into a 1 L volumetric flask. Add 20 mL of methanol. Add approximately 500 mL of DIW and mix to dissolve the reagents. Once dissolved, make to volume with DIW. Invert to mix. Vacuum filter the solution through a 0.20 µm Nylon filter. Adjust to pH 8.7 with either 12% (v/v) NH_3 or 5M HCl. If possible, prepare fresh on the day of analysis.

5.2.17 LC Mobile Phases (Seaweed)

5.2.17.1 20 mM Ammonium Carbonate in 2% Methanol, pH 8.7 (Mobile Phase A): Weigh 1.9212 g of ammonium carbonate into a 1 L volumetric flask. Add 20 mL of methanol. Add approximately 500 mL of DIW and mix to dissolve the reagents. Once dissolved, make to volume with DIW. Invert to mix. Vacuum filter the solution through a 0.20 µm Nylon filter. Adjust to pH 8.7 with either 12% (v/v) NH_3 or 5M HCl. If possible, prepare fresh on the day of analysis.

5.2.17.2 2% (v/v) Methanol (Mobile Phase B): Add 20 mL of methanol to a 1000 mL volumetric flask. Make to volume with DIW and invert to mix.

5.2.17.3 20 mM Ammonium Carbonate in 2% Methanol, pH 10.3 (Mobile Phase D): Weigh 1.9212 g of ammonium carbonate into a 1 L volumetric flask. Add 20 mL of methanol. Add approximately 500 mL of DIW and mix to dissolve the reagents. Once dissolved, make to volume with DIW. Invert to mix. Vacuum filter the solution through a 0.20 µm Nylon filter. Adjust to pH 10.3 with concentrated NH_3. If possible, prepare fresh on the day of analysis.

6 SAFETY PRECAUTIONS

6.1 Normal laboratory safety precautions are followed to ensure a safe and healthy environment, including the use of personal protective equipment (PPE). PPE will include, but not be limited to, a lab coat, protective eye wear and nitrile gloves.

6.2 The instrument room and laboratory environment can be very noisy; hearing protection is advised to avoid hearing loss from long term exposure.

6.3 The chemicals in this method are hazardous. Some compounds are known human carcinogens, others can cause burns or present inhalation hazards. Analysts should carefully read the Material Safety Data Sheet (MSDS) for each chemical used in this method. A powder hood can be used for the weighing of powdered chemicals. Consult applicable Safe Work Practice and Job Hazard Analysis Documents for further information.

6.4 ICP-MS instrumentation has high voltages, temperatures, levels of UV radiation and IR light present. Analysts must take precautions to avoid contact or exposure to these hazards.

6.5 Always add concentrated acid slowly into water when diluting.

6.6 Liquid Nitrogen is hazardous. When operating the Freezer/Mill for sample preparation, refer to Safe Work Practice #19.

6.7 For additional precautions to consider when performing this method refer to the CFIA Laboratory Safety Manual, the SOM-DAR-CHE-053 Job Hazard Analysis (JHA) and the associated Safe Work Practices (SWP's).

7 POLICY

7.1 Actual Stock Standard solution concentrations shall be calculated from the mass of primary standard, incorporating correction factors to account for the purity of the primary standard as well as arsenic equivalency factors. Solution concentrations given for subsequent solutions shall be considered nominal and actual concentration shall be calculated from the corrected concentration of the stock standard.

7.2 Perishable samples shall be kept frozen as much as possible to reduce the possibility of arsenic interspecies conversion. Preferably, samples are received fresh, prepared as quickly as possible, and then frozen.

7.3 Instrument parameters given in this method shall be used as guidelines only and may vary slightly between instruments and/or LC columns. Analysts shall refer to the instrument manual and other materials provided by the manufacturer for additional safety and instrument operation information.

7.4 If results are non-compliant with applicable regulations or other assessment criteria provided to the laboratory by the client, the result(s) shall be confirmed by re-running the sample. If spiking the sample, ensure the amount of spike solution added is suitable for the level of analyte found in the initial sample analysis.

7.5 Until a certified reference material (CRM) for speciated arsenic becomes available, spiked samples, reference materials, or an internally prepared check sample shall be used for QA purposes.

7.6 Results shall be corrected using spike recoveries when the recovery is outside a 90% to 110% range.

8 INSTRUCTIONS

8.1 General Sample Preparation

8.1.1 Take precautions to prevent contamination of the sample from materials on the outside of the packaging. Thoroughly scrub the cutting board, knife(s), spatula, blender bowls, etc. and immediate work area to minimize cross-contamination between samples.

8.1.2 If samples consist of multiple sub-samples, homogenize the entire sample to make a composite or homogenize each sub-sample individually and take equal amounts to make a composite, retaining the individuals if necessary.

8.1.2.1 Non-liquid foods (e.g. cereals, fruit, canned food): Blend the edible portion of the product in a blender or food processor at the highest speed possible until homogenized. Avoid grinding samples so long that the food processor begins to generate excessive heat. Transfer homogenate to a clean sample storage container.

8.1.2.2 Liquid samples: Vigorously shake or stir the sample until homogenous. Immediately pour into a clean sample storage container.

8.1.2.3 Sugary foods and Seaweeds: Freeze the product for 2 hours in a plate freezer. Transfer the food product to a -80ºC freezer and leave overnight. Blend the sample as soon as possible once it has been removed from the freezer so that the product does not stick to the blender bowl or blades. Transfer the homogenate to a clean sample storage container. Alternatively, use the Spex 6970 EFM Freezer/Mill to grind the samples. Samples are packed in specifically designed holders and placed in the unit where they are

frozen (using liquid nitrogen) and pulverized. Transfer the homogenate to a clean sample storage container.

8.1.3 Freeze perishable sample homogenate(s) in a sealed container until analysis. At the time of analysis, ensure the prepared sample composite is homogenous prior to weighing the portion for extraction. If liquid separates on thawing, mix well or re-blend prior to use.

8.2 Rinse extraction vessels (50 mL PP centrifuge tubes or Digitubes) and caps three times with DI water and invert to dry. Liquid food samples (e.g. rice beverage) shall be extracted in Digitubes and all other matrices in PP tubes. Rinse a set of PP centrifuge tubes and Digitubes for seaweed samples. Water and juice samples shall be extracted in Falcon tubes (See QA/QC Considerations 9.6 for additional information).

8.3 Extraction:

Note: See 8.3.1 for enzymatic digestion (all matrices other than water and juice). See 8.3.2 for water samples. See 8.3.3 for juice samples.

8.3.1 Enzymatic digestion

8.3.1.1 Weigh samples into appropriate tubes. Use Table 1 in Appendix 1 as a guide for sample weight.

8.3.1.2 For each set of samples include a reagent blank, a sample to be run in duplicate and spiked for each matrix type, and if available a CRM or reference material. A typical spike volume is 0.025 mL of the General Spiking Solution.

8.3.1.3 Calibration Curve Tubes: Refer to Table 1 in Appendix 1 as a guide for type of calibration curve to be used.

8.3.1.3.1 Reagent blank matched curve: Prepare four additional tubes. Follow steps 8.3.1.4 – 8.3.1.7.

8.3.1.3.2 Matrix matched curve: Prepare four additional tubes. Weigh a sample (i.e. being analyzed in the run) into each tube and follow steps 8.3.1.4 – 8.3.1.7.

8.3.1.3.3 NIST 1568a curve: If reference material NIST 1568a is being analyzed for QA/QC purposes an additional curve is required for extractions with matrix matched curves. Prepare an extra two tubes and follow steps 8.3.1.4 – 8.3.1.7.

8.3.1.4 Add 0.5 mL of protease solution to each sample tube. (The concentration of protease solution used will depend on the estimated protein content of the sample. Use Table 1 in Appendix 1 as a guide to determine the appropriate protease solution to be used).

8.3.1.5 Add 1.0 mL of lipase/alpha-amylase solution to each sample tube.

8.3.1.6 Add Extraction solution

8.3.1.6.1 For liquid food samples extracted in Digitubes make up to 15 mL mark. Vortex for 10 seconds.

8.3.1.6.2 For all other sample matrices (in PP tubes) add 13.5 mL to each sample tube. Vortex for 10 seconds.

8.3.1.7 Rotate tubes in an incubator at 37ºC for a minimum of 16 hours.

8.3.1.8 Matrices other than seaweed:

8.3.1.8.1 After incubation centrifuge tubes at 3000 rpm (2094 x g) for 10 minutes. Transfer samples to micro-centrifuge tubes if using Digitubes and centrifuge at 14000 rpm (16000 x g) for 10 minutes.

8.3.1.8.2 Filter extracts through 13 or 25 mm, 0.2 µm syringe filter into an autosampler vial.

8.3.1.9 Seaweed:

8.3.1.9.1 Centrifuge tubes at 3000 rpm (2094 x g) for 10 minutes.

8.3.1.9.2 Transfer the supernatant to a 50 mL Digitube.

8.3.1.9.3 Add 15 mL of extraction solution to the sample. Vortex for 10 seconds. Centrifuge tubes at 3000 rpm (2094 x g) for 10 minutes. Transfer the supernatant to the tube with first supernatant.

8.3.1.9.4 Repeat 8.3.9.1.3.

8.3.1.9.5 Make the combined supernatants up to 50 mL mark with extraction solution.

8.3.1.9.6 Filter extracts through 13 or 25 mm, 0.2 µm syringe filter into an autosampler vial.

8.3.2 Water Samples

8.3.2.1 For each set of samples include a reagent blank, and at least two samples to be run in duplicate and spiked, and if available a CRM or reference material.

8.3.2.2 Pipette 7.5 ml of sample into a 14 mL Falcon tube.

8.3.2.3 Fortify sample(s) with an aliquot of the Water/Juice Spiking Solution. A typical spike volume is 0.025 mL.

8.3.2.4 Add 2.5 mL of methanol to tubes. Mix.

8.3.2.5 Pour an aliquot into an autosampler vial.

8.3.3 Juice Samples

8.3.3.1 For each set of samples include a reagent blank, a sample to be run in duplicate and spiked for each matrix type, and if available a CRM or reference material.

8.3.3.2 Pipette 2.0 mL of sample into a 50 mL PP tube.

8.3.3.3 Fortify sample(s) with an aliquot of the Water/Juice Spiking Solution. A typical spike volume is 0.025 mL.

8.3.3.4 Add 6.0 mL of 25% MeOH to tubes. Vortex.

8.3.3.5 Filter an aliquot through 13 or 25 mm, 0.2 µm syringe filter into an autosampler vial.

8.4 Calibration Standards Preparation:

8.4.1 For reagent blank matched and matrix matched curves combine the supernatant of the four calibration curve tubes into a pre-rinsed tube, to be used as diluent.

8.4.1.1 Transfer the volumes of working standard solutions as indicated in Table 4 into 14 mL Falcon tubes.

8.4.2 For reference material NIST 1568a curve combine the supernatant of the two calibration curve tubes into a pre-rinsed tube, to be used as diluent.

8.4.2.1 Transfer the volumes of working standard solutions as indicated in Table 4 for Standards 0, 2 and 4 into 14 mL Falcon tubes.

8.4.3 For water and juice samples transfer the volume of working standard solution as indicated in Table 4 into 14 mL Falcon tubes. Use 25% MeOH as diluent.

8.4.4 Filter calibration standards through 13 or 25 mm, 0.2 µm syringe filter into an auto sampler vial.

Table 4: Guide for Preparation of Arsenic Speciation Calibration Standards

Standard	Volume of Low Level As^{+3}/DMA Working Standard (mL)	Volume of High Level As^{+3}/DMA Working Standard (mL)	Volume of As^{+5}/AsC/AsB Working Standard (mL)	Volume of MMA Working Standard (mL)	Volume of Matrix Diluent (mL)
0	0.000	-	0.000	0.000	10.000
1	0.025	-	0.025	0.010	9.940
2	0.100	-	0.050	0.040	9.810
3	-	0.100	0.100	0.200	9.600
4	-	0.500	0.500	0.800	8.200

Note: Refer to Appendix 1, Table 2 for Standard Concentrations.

8.5 LC-ICP-MS Analysis

8.5.1 Allow the LC and ICP-MS to equilibrate by running to waste for ~ 30 minutes prior to use. The ICP-MS may require additional equilibration time.

8.5.2 Check the ICP-MS performance according to manufacturer's specifications. See Appendix 3 for suggested ICP-MS and LC operating conditions.

8.5.3 Load the auto sampler. Initiate analysis as per manufacturer's instructions. Ensure the output from the LC will either calculate or allow for measurement of analyte peak response.

8.6 Calculation and Expression of Results

8.6.1 Prepare a calibration curve by using instrument response and the concentration (ng/mL) of calibration working standards for each arsenic species.

8.6.1.1 Convert compounds to arsenic equivalents, e.g. 1000 ng/mL Cacodylic Acid ($(CH_3)_2As(O)OH$) would be equivalent to 532 ng/mL As equivalents.

$$\frac{74.9 \text{ g/mol (elemental mass of arsenic)}}{138.0 \text{ g/mol (molecular mass of DMA)}} = 0.543$$

0.543 x 1000 ng/mL = 543 ng/ml

543 ng/mL (98% Purity) = 543 ng/mL x 0.98 = 532 ng/mL

8.6.1.2 Plot peak response against concentration using the linear regression equation y = mx + b where,

x = standard concentration in arsenic equivalents (ng/mL)
y = instrument response (peak response)
m = slope of calibration curve
b = intercept (0 - force through origin)

8.6.2 For all samples except water and juice: Calculate the final concentration (ng/mL) of arsenic equivalents in the sample extract using the equation above (8.6.1.2) to solve for x; x = (y-b)/m, where y is the response of the sample and solving for x will give the sample extract concentration. Calculate the concentration (ng/g) of arsenic equivalents in the sample using the formula below:

$$\text{Sample conc. (ng/g)} = \frac{\text{Extract Conc. (ng/mL) x Final Volume of Extract (mL)}}{\text{Mass of Sample (g)}}$$

8.6.3 For waters and juice samples, calculate the final concentration (ng/mL) of arsenic equivalents in the sample extract using the equation above (8.6.1.2) to solve for x; x = (y-b)/m, where y is the response of the sample and solving for x will give the sample extract concentration. Calculate the concentration (ng/mL) of arsenic equivalents in the sample using the formula.

$$\text{Sample conc. (ng/mL)} = \frac{\text{Extract Conc.(ng/mL) x Final Volume of Extract (mL)}}{\text{Volume of Sample (mL)}}$$

8.6.4 All data (responses for calibration standards, reagent blanks, samples, and check standards) will be entered into excel templates. The final concentrations will be calculated automatically using the linear estimate and the built-in formulas found in 8.6.2 and 8.6.3. Table 5 lists the RDIMS numbers associated with the excel template used to calculate

the data. The templates are in a "read-only" format. The latest version of the template shall be the one used for spreadsheet calculations.

Table 5: RDIMS file numbers for calculation templates

Matrix	RDIMS #
Rice-based Products Single-grain Cereals	3158952
Fruit-based Products Multi-grain Cereal Infant Formula Wheat & Rice Bran	3168239
Seaweed	3277051
Water	3168272
Juice	3488285

9 QA/QC CONSIDERATIONS

9.1 The LC mobile phases should be used within a couple of days of their preparation. Ammonium carbonate is extremely prone to mould growth which will have an adverse effect on the instrument's injector, sample loop, switching valve, nebuliser, guard column and column.

9.2 The reproducibility of this method is determined by entering a control sample or certified reference material result in a quality control chart (MS Excel file) designed for this analysis. For each analytical run, the quality of the results is determined by assessing for compliance with the policy and procedures given in SOP-DAR-LAB-002 (Control Charts). Currently, there is no commercially available certified reference material for evaluation.

9.3 The linearity of the standard curve (r^2) must be greater than 0.950.

9.4 The amount of protease added will be adjusted according to the estimated protein content of the food. The amount of protease added will also take into consideration that Arsenic^{+5} is naturally present in the protease. (The samples are blank-corrected to account for this). The amount of protease to be added will be based on previous method development findings for matrices with similar protein content.

9.5 The decision of which sample weight is more suitable to the analysis will be based on the moisture content and the expected speciated arsenic concentration

in the sample (the expected concentrations can generally be found through literature searches). For example, a 2-gram subsample would be extracted for a matrix with high moisture content and low expected speciated arsenic concentrations such as is found in pear-based pureed baby food. In comparison, a 1-gram subsample would be extracted for a matrix with low moisture content and high expected speciated arsenic concentrations such as is found in rice-based products.

9.6 Samples with a high moisture content (e.g. rice beverage) are assumed to contribute to the final volume (15 mL) of the extraction, for these samples a Digitube shall be used and extraction solution added to make a final volume of 15 mL. Samples with a low moisture content (e.g. rice) will be extracted in 50 mL PP tubes. 13.5 mL of extraction solution will be added along with 1.0 mL of lipase/alpha-amylase solution and 0.50 mL of protease solution for a final volume of 15 mL.

9.7 If reference materials or samples with high-levels of naturally incurred arsenic species are submitted for analysis, higher levels of calibration standards will need to be prepared or a dilution will need to be performed. Note, in the laboratory notebook, the calibration standards prepared as well as the source standards used in their preparation or if any dilutions were carried out.

9.8 The analytical range and LODs/LOQs for single and multi-grain cereals and rice beverages are the same as those determined for infant rice cereal. The analytical range and LODs/LOQs for fruit-based products (e.g. candies, jams and sauces) are the same as those determined for pear-based pureed baby food).

9.9 Some types of seaweed samples (e.g. Nori) contain arsenosugars that will carry-over into the chromatography of the subsequent sample. To resolve this issue a wash may be run between samples or extend the gradient of the D mobile phase for samples containing large amounts of arsenosugars.

9.10 This method has been validated for the analysis of speciated arsenic in infant rice cereal, pear-based pureed baby food, and water. As the spectrum of matrices included under the scope of this method continues to expand, so does the need for validation/verification. However, it is not practical to carry out full validation/verification for each matrix of interest. It is assumed that matrices with similar characteristics (e.g. high water content, high starch content etc.) will behave similarly and that validation performance characteristics (e.g. LOD/LOQ) can be applied to samples in similar matrix categories. A method validation/verification shall be performed on matrices which do not fall within a previously validated/verified matrix category.

Appendix 1

Table 1: Sample Preparation Guide

Matrix	Mass (g)	Protease Solution Conc (mg/0.5mL)	Standard Curve
Rice-based Products (Dry), Single-grain Cereal	1 ± 0.5000	60	Reagent Blank Matched
Rice-based Products (Liquid)	1 ± 0.5000	60	Matrix Matched
Multi-grain Cereal, Wheat Bran, Rice Bran Infant Formula	1 ± 0.5000	60	Matrix Matched
Fruit Based Products	2 ± 0.5000	30	Matrix Matched
Seaweed	0.5 ±0.2000	60	Matrix Matched
Reference Material 1568a	0.4 ± 0.2000	60	Reagent Blank Matched
Water	-	-	25% MeOH
Juice	-	-	25% MeOH

Table 2: Calibration Standards Concentrations

Standard	As^{+3} (ng/mL)	DMA (ng/mL)	MMA (ng/mL)	AsC (ng/mL)	As^{+5} (ng/mL)	AsB (ng/mL)
0	0.000	0.000	0.000	0.000	0.000	0.000
1	0.500	0.250	0.694	0.500	0.350	0.250
2	2.000	1.000	2.778	1.000	0.700	0.500
3	20.00	10.00	13.89	2.000	1.400	1.000
4	100.0	50.00	55.55	4.000	7.000	2.000

Appendix 2

Table 1: Analytical Range in Arsenic Equivalents

Species	Infant Rice Cereal (ng/g)	Pear-Based Pureed Baby Food (ng/g)	Seaweed (ng/g)	Water (ng/mL)	Juice (ng/mL)
AsC	0.39 – 460	0.52 – 230	-	0.061 – 4.23	0.18 – 12.7
AsB	0.41 – 300	0.23 – 170	-	0.083 – 2.93	0.25 – 8.80
As^{+3}	0.68 – 1500	0.66 – 750	10 – 10000	0.078 – 133	0.24 – 400
DMA	0.70 – 410	0.36 – 240	5- 3080	0.043 – 41.0	0.13 – 123
MMA	0.98 – 860	0.58 – 435	13 – 2315	0.088 – 30.9	0.26 – 92.8
As^{+5}	4.80 - 1050	2.67 - 525	10 - 700	0.127 – 9.31	0.38 – 28

Appendix 3

The following are the suggested instrument operating conditions. Additional information may be found in the instrument manual and other materials provided by the manufacturer.

Table 1: ICP-MS parameters

RF Power	1350 W
Plasma Flow	15 L/min
Auxiliary Flow	1.2 L/min
Nebuliser Flow	0.7 - 1.0 L/min
Lens Voltage	7 - 10 V
Analyte Mass	AsO 90.9
Dwell Time	500 ms
Instrument Mode	DRC-B
Gas B (Oxygen) Mode	0.5 L/min
Detector Mode	Pulse

Table 2: HPLC parameters

	All Matrices Other than Seaweed	Seaweed
Column	PRP X-100 (250 mm x 4.0 x 10 µm) with guard	PRP X-100 (250 mm x 4.0 x 10 µm) with guard
Pump Setting	Gradient (see Table 3)	Gradient (see Table 4)
Flow rate	1.2 mL/min	1.0 mL/min
Run Time	13.5 minutes	30 minutes
Equilibration Time	0.1 minute between injections	0.1 minute between injections
Injection Volume	50 µL	50 uL
Column Temperature	25 ºC	25 ºC

Table 3: HPLC gradient for all matrices other than seaweed:

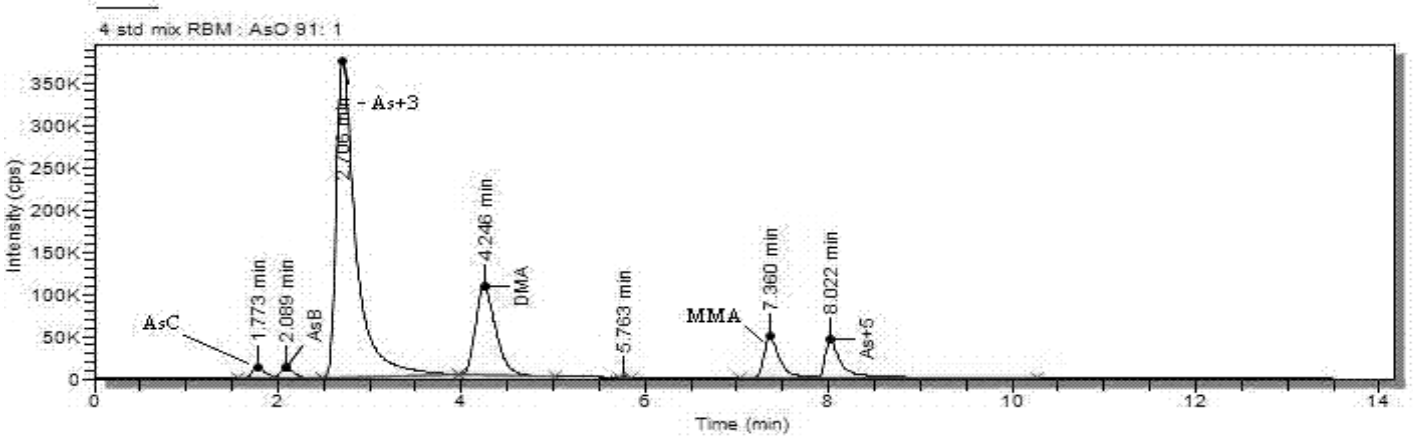
Step	Step Type	Step Time (min)	Flow (ml/min)	%A	%B	%C	%D	Curve
0	Equil	0.1	1.2	100	0	0	0	1
1	Run	2	1.2	100	0	0	0	1
2	Run	1	1.2	0	100	0	0	1
3	Run	6.5	1.2	0	100	0	0	1
4	Run	1	1.2	100	0	0	0	1
5	Run	3	1.2	100	0	0	0	1

Table 4: HPLC gradient for seaweed:

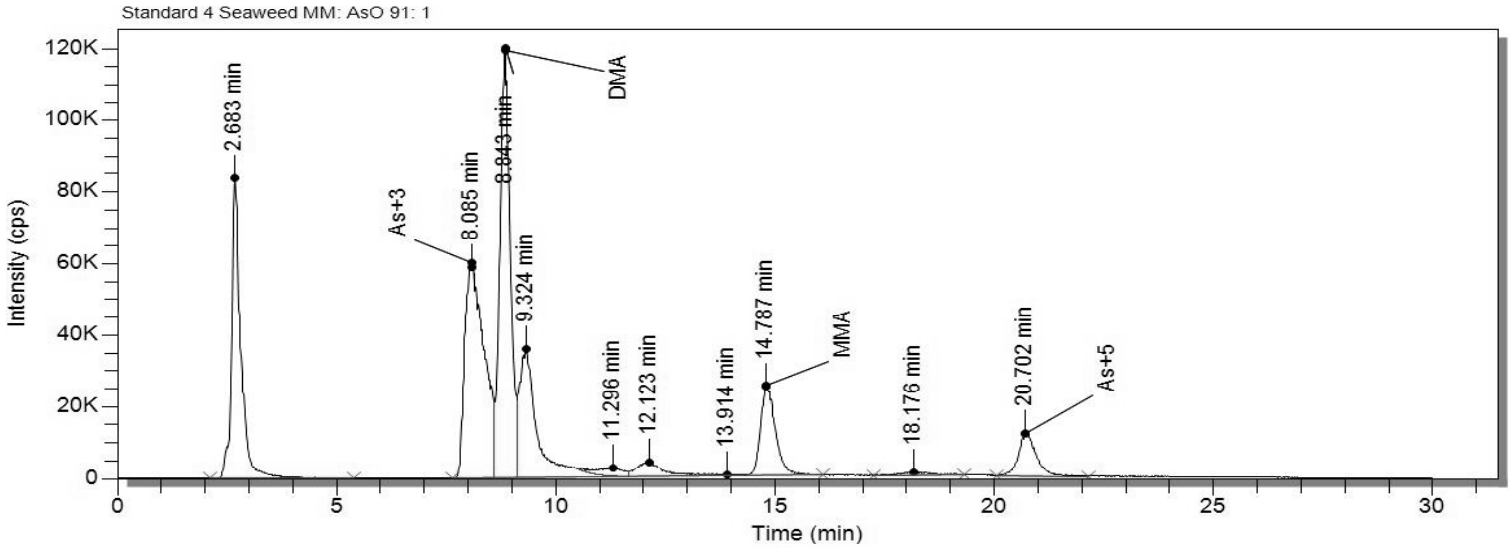
Step	Step Type	Step Time (min)	Flow (ml/min)	%A	%B	%C	%D	Curve
0	Equil	0.1	1.0	5	95	0	0	1
1	Run	3.5	1.0	5	95	0	0	1
2	Run	0.1	1.0	100	0	0	0	1
3	Run	10.4	1.0	100	0	0	0	1
4	Run	0.1	1.0	0	0	0	100	1
5	Run	8.9	1.0	0	0	0	100	1
6	Run	0.1	1.0	5	95	0	0	1
7	Run	6.9	1.0	5	95	0	0	1

Appendix 4

4 std mix RBM



Standard 4 Seaweed MM : AsO 91 : 1



Attachment 6 to Appendix 1, version 2

This document is CONFIDENTIAL when completed

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Standard Operating Document Title: Determination of α-Solanine and α-Chaconine in Potato Tubers

APPROVAL COVER PAGE

This cover page is proof of final approval of this standard operating document. Upon the issuance of a new version of the document a new approval page will be included. The obsolete approval page will be archived with the master document of the obsolete document.

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Activation Date: 20120430

UNCONTROLLED

SOM-CHE-055-02

DETERMINATION OF α-SOLANINE AND α-CHACONINE IN POTATO TUBERS

1. PURPOSE

1.1. To provide specific instructions for the determination of α-Solanine and α-Chaconine in potatoes.

2. REFERENCES

2.1. AOAC Official Method 997.13 Glycoalkaloids (α-Solanine and α-Chaconine) in Potato Tubers

2.2. SOP-DAR-LAB-002 Control Charts

3. SCOPE

3.1. This procedure is applicable for quantitative determination of 10-200 mg/kg α-Solanine and 20-250 mg/kg α-Chaconine in raw potato tubers.

3.2. Glycoalkaloids are extracted from fresh tuber tissue with dilute acetic acid. The extract is concentrated and cleaned up on disposable solid phase extraction cartridges. Final separation and measurement of α-Solanine and α-Chaconine is done by reverse-phase liquid chromatography with ultra violet detection at 202 nm.

3.3. Only trained analysts shall perform this analysis.

4. DEFINITIONS

4.1. LOD - Limit of Detection

4.2. LOQ - Limit of Quantification

4.3. MeCN - Acetonitrile

4.4. SPE - Solid Phase Extraction

5. EQUIPMENT AND MATERIALS REQUIRED

5.1. Equipment

5.1.1. Robot Coupe food processor or equivalent

5.1.2. pH meter

5.1.3. Sample cups

5.1.4. 50 mL polypropylene tubes

5.1.5. Balance capable of ∀0.01 gram accuracy

5.1.6. Wrist action rotator

5.1.7. Centrifuge capable of 4000 g.

5.1.8. Funnels

5.1.9. Glass wool

5.1.10. Autosampler vials and caps

5.1.11. Sep Pak Plus SPE cartridges

5.1.12. Vacuum manifold

5.1.13. 6 mL SPE reservoirs

5.1.14. 15 mL disposable tubes

5.1.15. 15 mL graduated centrifuge tubes with stoppers

5.1.16. Vortex mixer

5.1.17. Nylon syringe filters, 0.2 μm pore size or equivalent

5.1.18. Ultra high performance liquid chromatography System

5.1.18.1. LC Pump System capable of providing flow rates up to 3.0 mL/min and at pressures of at least 15000 psi.

5.1.18.2. Autosampling system able to communicate with the pump and data system and providing injection volumes up to 20 μl.

5.1.18.3. Column oven capable of maintaining column temperatures of 60^{o}C

5.1.18.4. LC Column: Waters BEH C18 or equivalent

5.1.18.5. UV detector capable of providing the required sensitivity at 202 nm.

5.2. Reagents

5.2.1. Acetonitrile (MeCN) - LC grade

5.2.2. Deionized water (DIW), 18.0 MΩ-cm or equivalent

5.2.3. Glacial acetic acid

5.2.4. Sodium bisulfite ($NaHSO_3$)

5.2.5. Anhydrous dipotassium phosphate (K_2HPO_4)

5.2.6. Potassium dihydrogen phosphate (KH_2PO_4)

5.2.7. Extraction Solution: DIW - Glacial acetic acid - $NaHSO_3$ (100 + 5 + 0.5, v/v/w). Mix 1.0L DIW with 50 mL of glacial acetic acid, add 5.0 g $NaHSO_3$ and mix.

5.2.8. SPE Wash Solution: 15% MeCN - Measure 150 mL MeCN, make final volume 1.0 L with DIW and mix.

5.2.9. 0.1 M K_2HPO_4: Accurately weigh 17.4 g of anhydrous K_2HPO_4, quantitatively transfer to a 1.0 L volumetric flask; dissolve, bring up to volume with DIW and mix.

5.2.10. 0.1 M KH_2PO_4: Accurately weigh 13.6 g of KH_2PO_4, quantitatively transfer to a 1.0 L volumetric flask; dissolve, bring up to volume with DIW and mix.

5.2.11. 0.1 M Potassium phosphate buffer: Transfer ~100mL of 0.1 M K_2HPO_4 solution to a beaker with magnetic stirrer and a pH electrode. Adjust to pH 7.6 ∀ 0.01 with 0.1 M KH_2PO_4 solution (approx. 19 mL) while mixing.

5.2.12. LC Mobile Phase: 60% MeCN with 0.1 M Potassium phosphate buffer. Mix 100 mL of phosphate buffer solution with 600 mL MeCN, make final volume 1.0 L with DIW and mix.

5.2.13. LC Wash Solution: 60% MeCN: Measure 600 mL MeCN, make final volume 1.0 L with DIW and mix.

5.2.14. α-Solanine: powdered α-solanine from potato sprouts from Sigma Aldrich or equivalent

5.2.15. α-Chaconine: powdered α-chaconine from ABCR or equivalent

6. SAFETY PRECAUTIONS

6.1. Follow normal laboratory practices for a healthy and safe work environment.

6.2. α-Solanine and α-Chaconine are toxic substances. Wear a dust mask or work in a powder hood when working with powdered compounds.

6.3. Refer to MSDSs for all chemicals listed in this SOM.

7. POLICY

7.1. Actual Stock Standard solution concentrations shall be calculated from the mass of primary standard, incorporating correction factors to account for the purity of the primary standard. Solution concentrations given for subsequent solutions shall be considered nominal and actual concentrations shall be calculated from the corrected concentrations of the Stock Standard.

7.2. Quantitative transfer shall be an acceptable option for transferring a known amount of fine powder.

7.3. Volumes/masses listed in the instructions shall be used as guidelines when preparing solutions, but other volumes/masses may be used to create a solution with the same concentration.

8. INSTRUCTIONS

8.1. Preparation of Standard Solutions

8.1.1. Stock Standard Solutions:

8.1.1.1. α-Solanine stock (200 µg/mL): Accurately weigh to the nearest 0.05 mg, 5 mg of powdered α-solanine, bring up to final volume of 25 mL in a volumetric flask with 0.1M KH_2PO_4 and mix.

8.1.1.2. α-Chaconine stock (200 µg/mL): Accurately weigh to the nearest 0.05 mg, 5 mg of powdered α-chaconine, bring up to final volume of 25 mL in a volumetric flask with 0.1M KH_2PO_4 and mix.

8.1.2. Spiking solution (200 µg/mL α-solanine and 200 µg/mL α-chaconine): Accurately weigh to the nearest 0.05 mg, 5 mg of powdered α-chaconine and α-solanine, bring up to final volume of 25 mL in a volumetric flask with 0.1M KH_2PO_4 and mix.

8.1.3. Calibration curve (Table 1):

8.1.3.1. Prepare mixed working solutions with concentrations of 5, 10, 25, 50 and

100 µg/mL α-solanine and α-chaconine according to Table 1 in 2.0 mL volumetric flasks. Add specified volumes of α-solanine and α-chaconine, then add specified volume of MeCN without mixing, and make to volume as specified in Table 1. Mix solutions.

Table 1: Volumes of Stock Standards and reagents that are required to make standard curve solutions

Concentration	Volume to add (µL)		Volume MeCN (µL)	Volume of LC Mobile Phase
µg/mL	α-Solanine	α-Chaconine		
5	50	50	150	Make to final volume of 2.0 mL
10	100	100	300	Make to final volume of 2.0 mL
25	250	250	750	Make to final volume of 2.0 mL
50	500	500	Make to final volume of 2.0 mL	---
100	1000	1000	---	---

8.2. Preparation of Test Samples

8.2.1. Hand wash excess dirt off the potatoes with water, cut up into smaller pieces and blend 10-20 potato tubers in a food processor, until homogenous. Transfer homogenate to duplicate plastic containers with lids and store at #-18^{o}C.

8.3. Extraction

8.3.1. Completely thaw samples and thoroughly mix the sample.

8.3.2. Immediately weigh 5 g accurately into a 50 mL polypropylene tube.

8.3.3. Immediately add 20 mL of extraction solution.

8.3.4. Fortify specified samples at this time using 1000 µL of the spiking solution.

8.3.5. Mix on a wrist action rotator for 10 min.

8.3.6. Centrifuge tubes for 30 min at 4000 g.

8.3.7. Filter supernatant through a glass wool plug into a suitable vessel (sample extract

is stable for up to a week at 4°C) .

8.4. SPE Clean-up of Extract

8.4.1. Place SPE column onto vacuum manifold and condition each with 5.0 mL of MeCN followed by 5.0 mL of extraction solution - discard eluate.

8.4.2. Transfer 10.0 mL of sample extract to a glass 15 mL tube.

8.4.3. Load sample extract onto SPE columns and elute - discard eluate.

8.4.4. Rinse 15 mL tube with 4.0 mL SPE wash solution and load wash onto SPE column and elute - discard eluate.

8.4.5. Rinse 15 mL tube with 4.0 mL of LC Mobile Phase and load onto SPE column, collect eluate into a 15 mL graduated glass tube (elution rate: 1-2 drops/sec) .

8.4.6. Adjust volume to 5.0 mL with LC Mobile Phase, cap and vortex (eluate is stable for 1 week at 4°C) .

8.4.7. Filter extract through a 13 mm nylon syringe filter (0.2 µm pore size) into an auto sampler vial and cap.

8.5. UHPLC Conditions

8.5.1. Operate UHPLC as per manufacturer=s instructions.

8.5.2. Initial Setup and Conditions:

8.5.2.1. Allow system to start-up and flow with the conditions described below for approximately 20 minutes before each analytical run.
Column: Waters7 UPLC BEH C18 1.7 µm, 2.1 x 50 mm
Column Temperature: 60°C
Mobile Phase: 60% MeCN with 0.1 M Potassium phosphate buffer (5.2.12)
Wash Mobile Phase: 60% MeCN (5.2.13)
Flow Rate: 0.6 mL/min
Injection Volume: 2 µL
Detector - PDA λ 202 nm

8.5.3. Flow Parameters:

8.5.3.1. Use isocratic flow with the prepared mobile phase in 8.5.2.1.

8.5.3.2. Flush the column with the wash mobile phase (8.5.2.1) for at least 2 hours, using low flow (# 0.1 mL/ min), after each analytical run.

8.5.4. Run Considerations:

8.5.4.1. Inject an aliquot (2 μL) of standards, samples, spiked samples and reference materials on to the instrument. Identify peaks in samples by comparison of the retention times to that of the standards. Compare the resultant peak responses to response factors obtained from the appropriate calibration curve.

8.5.4.2. Dilute any samples that produce peak responses greater than the response for the most concentrated calibration solution for each specific toxin until the peak response falls within the calibration curve. Factor this dilution into concentration calculations.

8.6. Calculations

8.6.1. Prepare a standard curve by plotting the standard concentration (μg/mL) versus instrument response. Use the linear regression equation Y = mX + b, where Y is response, X is standard concentration (Φg/mL), m is the slope of the calibration curve and b is the intercept.

8.6.2. Use the following calculation to determine the sample concentrations from the standard curve. Correct for dilutions, mass of sample extracted and percent recovery from fortified samples.

8.6.3.

$$mg/100g = \left(\frac{\left(\frac{\mathbf{RSm}}{\mathbf{LinEst}}\right) \times \left(\frac{\mathbf{Wt + 20}}{\mathbf{Wt}}\right) \times \left(\frac{\mathbf{5}}{\mathbf{10}}\right)}{\mathbf{10}} \right) \div \%\text{Recovery}$$

Where: Rsm = peak response of the sample
LinEst = Linear Estimate of calibration curve
Wt = weight of sample
% Recovery = the percentage recovered from the fortified sample

9. QA/QC Considerations

9.1. The LC Conditions given should be used as guidelines and may differ slightly on each individual instrument.

9.2. Linearity of the standard curve (r^2) must be greater than 0.950.

9.3. LOD for this method is 0.2 mg/100g and LOQ is 0.6 mg/100g.

9.4. The reproducibility of the method (r) is determined by entering check sample or CRM results in the quality control chart (MS Excel file) designed for this analysis. Each analytical run is assessed for compliance with the policies and procedures given in SOP-DAR-LAB-002 Control Charts to determine the quality of the results.

9.5. Expected spike recoveries are shown in Table 2.

9.6. Analyse tissues subjected to a minimal number of freeze thaw cycles by using the second container of tissue homogenate to perform any confirmatory analyses if possible. Freeze/thaw cycles can increase starches and have a negative impact on chromatography.

Table 2: Results from comparison analyses of replicate spiked potato puree

	%Recovery ± %RSD	
Spike Level (mg/100g)	Solanine	Chaconine
10	90 ± 11	97 ± 10
20	82 ± 12	88 ± 11
40	85 ± 12	90 ± 11

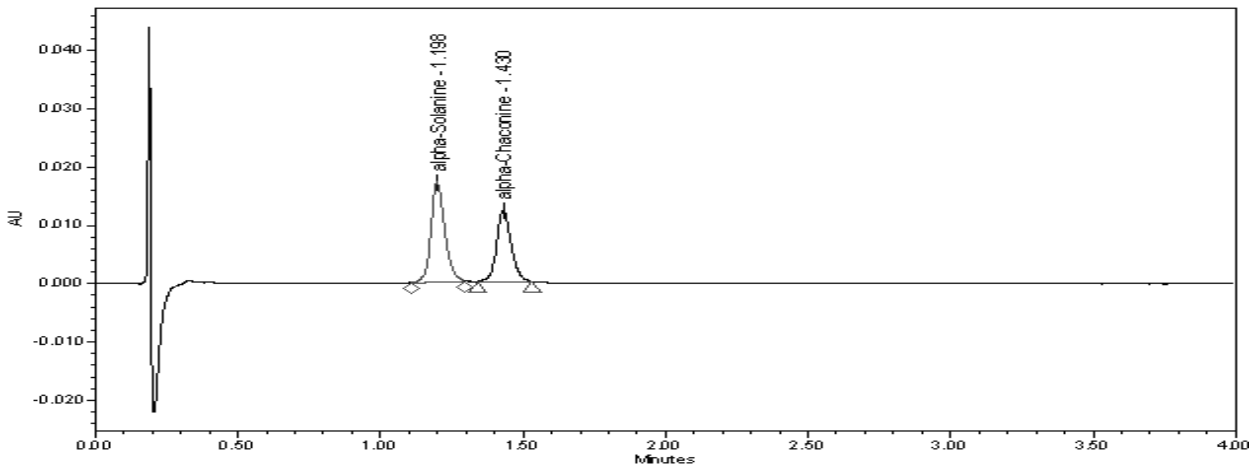

Figure 1: Chromatogram of 10 µg/mL Standard showing expected retention time and peak shape based on the conditions set in section 8.4

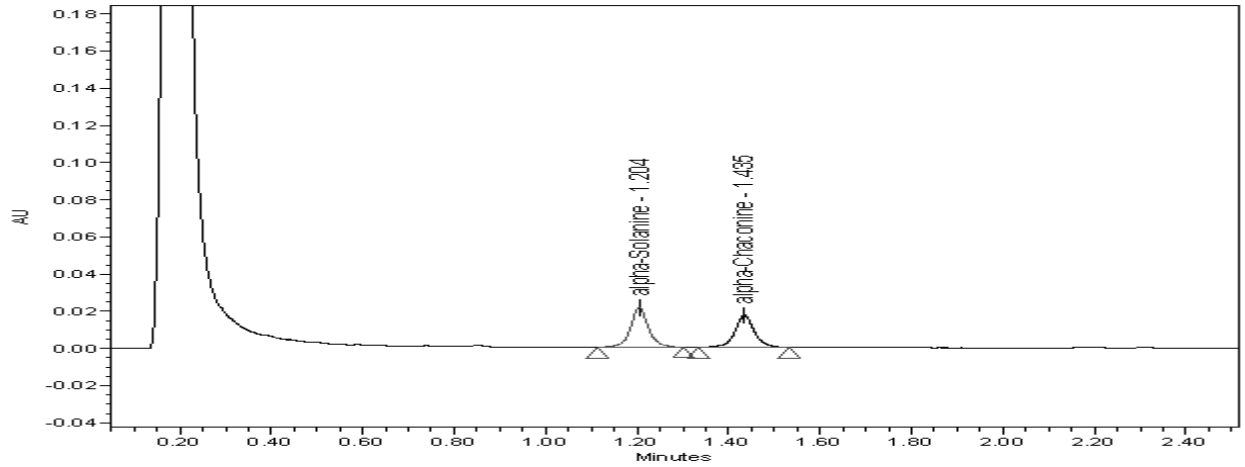

Figure 2: Example of a high level naturally incurred sample (15.2 mg/100g α-Solanine and 17.9 mg/100g α-Chaconine; Total = 33 mg/100g)

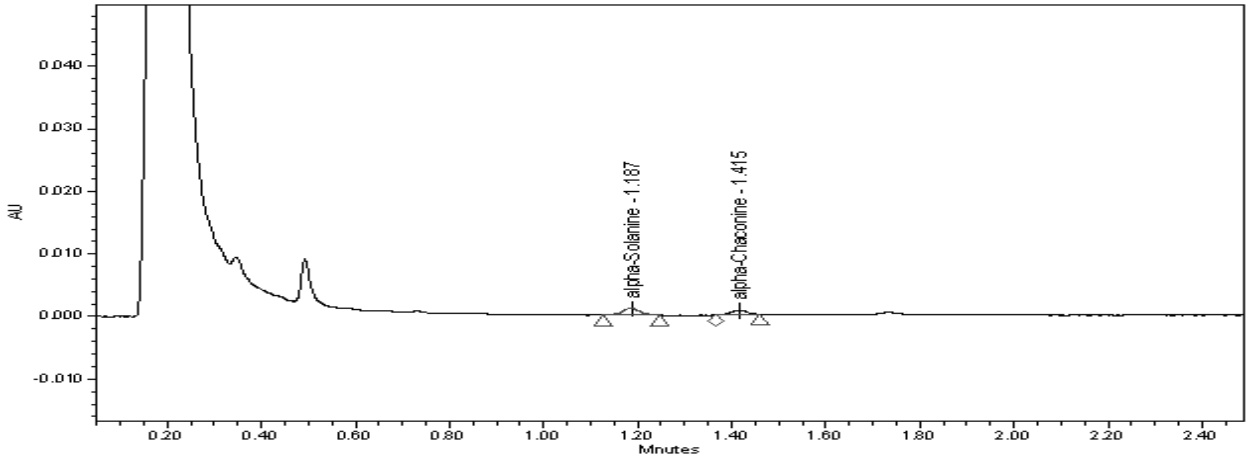

Figure 3: Example of a low level sample (0.7 mg/100g α-Solanine and 0.5 mg/100g α-Chaconine; Total = 1.2 mg/100g)