Public Works and Government Services Canada

Travaux publics et Services gouvernementaux Canada

RETURN BIDS TO:
RETOURNER LES SOUMISSIONS À:

Bid Receiving - PWGSC / Réception des soumissions - TPSGC
11 Laurier St. / 11, rue Laurier
Place du Portage , Phase III
Core 0A1 / Noyau 0A1
Gatineau, Québec K1A 0S5
Bid Fax: (819) 997-9776

REQUEST FOR PROPOSAL
DEMANDE DE PROPOSITION

Proposal To: Public Works and Government Services Canada

We hereby offer to sell to Her Majesty the Queen in right of Canada, in accordance with the terms and conditions set out herein, referred to herein or attached hereto, the goods, services, and construction listed herein and on any attached sheets at the price(s) set out therefor.

Proposition aux: Travaux Publics et Services Gouvernementaux Canada

Nous offrons par la présente de vendre à Sa Majesté la Reine du chef du Canada, aux conditions énoncées ou incluses par référence dans la présente et aux annexes ci-jointes, les biens, services et construction énumérés ici sur toute feuille ci-annexée, au(x) prix indiqué(s).

Comments - Commentaires

Title - Sujet
CHEMISTRY TESTING

Solicitation No. - N° de l'invitation	**Date**
39903-130313/A	2013-02-15

Client Reference No. - N° de référence du client
39903-130313

GETS Reference No. - N° de référence de SEAG
PW-$$SS-013-25446

File No. - N° de dossier	**CCC No./N° CCC - FMS No./N° VME**
013ss.39903-130313	

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:	**Buyer Id - Id de l'acheteur**
Dagenaig, Gaëtane	054sq

Telephone No. - N° de téléphone	**FAX No. - N° de FAX**
(819) 956-1365 ( )	(819) 997-2229

Destination - of Goods, Services, and Construction:
Destination - des biens, services et construction:

Specified Herein
Précisé dans les présentes

Instructions: See Herein

Instructions: Voir aux présentes

Vendor/Firm Name and Address
Raison sociale et adresse du fournisseur/de l'entrepreneur

Delivery Required - Livraison exigée	**Delivery Offered - Livraison proposée**
See Herein	

Vendor/Firm Name and Address
Raison sociale et adresse du fournisseur/de l'entrepreneur

Telephone No. - N° de téléphone
Facsimile No. - N° de télécopieur

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**

Issuing Office - Bureau de distribution

Science Procurement Directorate/Direction de l'acquisition de travaux scientifiques
11C1, Phase III
Place du Portage
11 Laurier St. / 11, rue Laurier
Gatineau, Québec K1A 0S5

Canada

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PART 1 - GENERAL INFORMATION

1. Introduction

The bid solicitation document is divided into seven parts plus attachments and annexes as follows:

Part 1 General Information: provides a general description of the requirement;

Part 2 Bidder Instructions: provides the instructions, clauses and conditions applicable to the bid solicitation;

Part 3 Bid Preparation Instructions: provides bidders with instructions on how to prepare their bid;

Part 4 Evaluation Procedures and Basis of Selection: indicates how the evaluation will be conducted, the evaluation criteria that must be addressed in the bid, and the basis of selection;

Part 5 Certifications: includes the certifications to be provided;

Part 6 Insurance Requirements: includes specific requirements that must be addressed by bidders; and

Part 7 Resulting Contract Clauses: includes the clauses and conditions that will apply to any resulting contract.

The Annexes include the Statement of Work, the Basis of Payment, the Insurance Requirements and the Task Authorization Form.

2. Summary

(i) The Canadian Food Inspection Agency (CFIA) is a federal regulatory agency with a mandate to safeguard food, animals and plants to enhance the health and well-being of Canada's people, environment and economy.

In December 2007 the Government of Canada announced the Food and Consumer Safety Action Plan (FCSAP), a comprehensive set of proposed new measures that will make Canadians safer by legislating tougher federal government regulation of food, health and consumer products. The Food Safety Action Plan (FSAP) was one element of that broader plan focusing on those products considered as "non-federally registered" sector and conservatively makes up 70 percent of the food Canadians consume.

The FSAP encompassed a series of initiatives to modernize and strengthen Canada's food safety system over a period of five years. To attain a better understanding of the food safety risks that Canadians may be exposed to, the CFIA needs to conduct surveys to determine the background levels of contamination in certain food areas.

In addition, the CFIA may be required to take regulatory action under any or all of the Acts it administers or enforces by virtue of section 11 of the Canadian Food Inspection Agency Act, or under any other applicable law, on the basis of any information received or obtained in the course of performing the Work under this contract.

(ii) The CFIA is looking for commercial laboratories to provide sample collection and analytical testing services in food products. The results of the testing will be used by CFIA to determine the food safety risk to Canadians and identify areas where food safety issues may need to be addressed.

(iii) Sample collection and analytical testing services of food are required from May 1, 2013 to April 30, 2016, with an irrevocable option on the part of Canada to extend the contract period by up to two (2) optional periods of one (1) year. A portion of the Work in the contract(s) that includes Optional Survey Packages, Expert Testimony Services, Additional Surveys Services if applicable, and Additional Analytical Testing Serivces, will be on an "as and when requested" basis subject to Task Authorizations.

(iv) Canada intends to award up to fifteen (15) contracts to ensure Laboratory Services are available for all Surveys as identified in Annex A, Statement of Work, however only one contract per Survey will be issued based on the results of the financial evaluation. If a Bidder is recommended for award of a Contract for more than one Survey, the Contract issued will cover all the applicable Surveys to the Bidder.

(v) The requirement is subject to the provisions of the Agreement on Internal Trade (AIT).

(vi) The requirement is limited to Canadian services as defined in paragraph 2 of SACC Manual clause A3050T.

3. **Debriefings**

After contract award, bidders may request a debriefing on the results of the bid solicitation process. Bidders should make the request to the Contracting Authority within ten (10) working days of receipt of the results of the bid solicitation process. The debriefing may be in writing, by telephone or in person.

PART 2 - BIDDER INSTRUCTIONS

1. Standard Instructions, Clauses and Conditions

All instructions, clauses and conditions identified in the bid solicitation by number, date and title are set out in the *Standard Acquisition Clauses and Conditions Manual* (*https://buyandsell.gc.ca/policy-and-guidelines/standard-acquisition-clauses-and-conditions-manual)* issued by Public Works and Government Services Canada.

Bidders who submit a bid agree to be bound by the instructions, clauses and conditions of the bid solicitation and accept the clauses and conditions of the resulting contract.

The 2003 (2012-11-19) Standard Instructions - Goods or Services - Competitive Requirements, are incorporated by reference into and form part of the bid solicitation.

Subsection 5.4 of 2003, Standard Instructions - Goods or Services - Competitive Requirements, is amended as follows:

Delete: sixty (60) days
Insert: one hundred and twenty (120) days

1.1 SACC Manual Clauses

A7035T (2007-05-25), List of Proposed Subcontractors

2. Submission of Bids

Bids must be submitted only to Public Works and Government Services Canada (PWGSC) Bid Receiving Unit by the date, time and place indicated on page 1 of the bid solicitation.

Due to the nature of the bid solicitation, bids transmitted by facsimile to PWGSC will not be accepted.

3. Enquiries - Bid Solicitation

All enquiries must be submitted in writing to the Contracting Authority no later than ten (10) calendar days before the bid closing date. Enquiries received after that time may not be answered.

Bidders should reference as accurately as possible the numbered item of the bid solicitation to which the enquiry relates. Care should be taken by bidders to explain each question in sufficient detail in order to enable Canada to provide an accurate answer. Technical enquiries that are of a proprietary nature must be clearly marked "proprietary" at each relevant item. Items identified as proprietary will be treated as such except where Canada determines that the enquiry is not of a proprietary nature. Canada may edit the questions or may request that the Bidder do so, so that the proprietary nature of the question is eliminated, and the enquiry can be answered with copies to all bidders. Enquiries not submitted in a form that can be distributed to all bidders may not be answered by Canada.

4. Applicable Laws

Any resulting contract must be interpreted and governed, and the relations between the parties determined, by the laws in force in Ontario.

Bidders may, at their discretion, substitute the applicable laws of a Canadian province or territory of their choice without affecting the validity of their bid, by deleting the name of the Canadian province or

territory specified and inserting the name of the Canadian province or territory of their choice. If no change is made, it acknowledges that the applicable laws specified are acceptable to the bidders.

5. Improvement of Requirement During Solicitation Period

Should bidders consider that the specifications or Statement of Work contained in the bid solicitation could be improved technically or technologically, bidders are invited to make suggestions, in writing, to the Contracting Authority named in the bid solicitation. Bidders must clearly outline the suggested improvement as well as the reason for the suggestion. Suggestions that do not restrict the level of competition nor favour a particular bidder will be given consideration provided they are submitted to the Contracting Authority at least ten (10) days before the bid closing date. Canada will have the right to accept or reject any or all suggestions.

6. Basis for Canada's Ownership of Intellectual Property

The Canadian Inspection Food Agency has determined that any intellectual property rights arising from the performance of the Work under the resulting contract will belong to Canada, on the following grounds:

- the main purpose of the contract, or of the deliverables contracted for, is to generate knowledge and information for public dissemination**.**

PART 3 - BID PREPARATION INSTRUCTIONS

1. Bid Preparation Instructions

Canada requests that bidders provide their bid in separately bound sections as follows:

Section I : Technical Bid (4 hard copies per Survey)
Section II : Financial Bid (2 hard copies)
Section III : Certifications (1 hard copy)

Prices must appear in the financial bid only. No prices must be indicated in any other section of the bid.

Canada requests that bidders follow the format instructions described below in the preparation of their bid:

(a) use 8.5 x 11 inch (216 mm x 279 mm) paper; and
(b) use a numbering system that corresponds to the bid solicitation.

In April 2006, Canada issued a policy directing federal departments and agencies to take the necessary steps to incorporate environmental considerations into the procurement process Policy on Green Procurement (*http://www.tpsgc-pwgsc.gc.ca/ecologisation-greening/achats-procurement/politique-policy-eng.html*). To assist Canada in reaching its objectives, bidders should:

(1) use paper containing fibre certified as originating from a sustainably-managed forest and containing minimum 30% recycled content; and

(2) use an environmentally-preferable format including black and white printing instead of colour printing, print double sided/duplex, using staples or clips instead of cerlox, duotangs or binders.

Bidders may bid on one or more Surveys detailed in Annex A, however, they should submit a separate technical bid for each Survey. Canada requests that bidders clearly identify on the front cover of their technical bid which Survey they are bidding on.

The category “Additional Survey(s)” described at article 11.4.3 in Annex A and item 6 of Attachment 1 to Part 3, Financial Bid Presentation Sheet is not mandatory. For bidders who are selected for contract award but who did not include a technical bid for “Additional Surveys”, the resultant contract will not include 11.4.1 in Annex A nor item 6 in the Basis of Payment.

Section I : Technical Bid

In their technical bid, bidders should demonstrate their understanding of the requirements contained in the bid solicitation and explain how they will meet these requirements. Bidders should demonstrate their capability and describe their approach in a thorough, concise and clear manner for carrying out the work.

The technical bid should clearly address and in sufficient depth the points that are subject to the evaluation criteria against which the bid will be evaluated. Simply repeating the statement contained in the bid solicitation is not sufficient. In order to facilitate the evaluation of the bid, Canada requests that bidders address and present topics in the order of the evaluation criteria under the same headings. To avoid duplication, bidders may refer to different sections of their bids by identifying the specific paragraph and page number where the subject topic has already been addressed.

Section II : Financial Bid

1.1 Bidders must submit their financial bid in accordance with the following:

(a) The information for each Survey as well as Optional Survey Package and Expert Testimony Services and Additional Surveys Services, if applicable, and Additional Analytical Testing Services should be provided on Attachment 1 to Part 3, Financial Bid Presentation Sheet.

(b) The total amount of Goods and Services Tax is to be shown separately, if applicable.

(c) For Canadian-based bidders, prices must be in Canadian funds, Canadian customs duties and excise taxes included, and Goods and Services Tax (GST) or Harmonized Sales Tax (HST) excluded.

For the purpose of the bid solicitation, bidders with an address in Canada are considered Canadian-based bidders and bidders with an address outside of Canada are considered foreign-based bidders.

Section III : Certifications

Bidders must submit the certifications required under Part 5.

PART 4 - EVALUATION PROCEDURES AND BASIS OF SELECTION

1. Evaluation Procedures

(a) Bids will be assessed in accordance with the entire requirement of the bid solicitation including the technical and financial evaluation criteria.

(b) An evaluation team composed of representatives of Canada will evaluate the bids.

(c) The evaluation team will only consider bids with a valid Canadian Content certification. If bids are received without a valid certification, they will be declared non-responsive and no further consideration will be given.

1.1 Technical Evaluation

1.1.1 Mandatory Technical Criteria

The mandatory technical criteria are described in Attachment 1 to Part 4, Mandatory Technical Criteria.

For the purpose of the mandatory technical criteria, the experience of the Bidder and its subcontractors will be considered as identified in Attachment 1 to Part 4, Mandatory Technical Criteria.

1.2 Financial Evaluation

1.2.1 Evaluation of Price

Bidders must submit their financial bid in accordance with Attachment 1 to Part 3, Financial Bid Presentation Sheet.

For evaluation purposes only, the evaluated price of the bid for each Survey including Expert Testimony Services and Optional Survey Packages, the evaluated price for Additional Surveys Services, if applicable, and the evaluated price for Additional Analytical Testing Services will be determined in accordance with Attachment 2 to Part 4, Evaluation of Price.

The price of the bid will be evaluated in Canadian dollars, the Goods and Services Tax or the Harmonized Sales Tax excluded, FOB destination, Canadian customs duties and excise taxes included.

2. Basis of Selection

2.1 Basis of Selection - Lowest Evaluated Price

2.1.1 To be declared responsive, a bid must:

(a) comply with all the requirements of the bid solicitation;

(b) meet all mandatory technical evaluation criteria.

Bids not meeting (a) or (b) will be declared non-responsive.

2.1.2 For each Survey:

Responsive bids will be ranked in ascending order of evaluated prices for each Survey, the responsive bid for each survey with the lowest evaluated price will be ranked first for each survey. 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. The responsive bid with the lowest evaluated price for each Survey will be

recommended for award of a contract. In the event that two or more responsive bids have the same lowest evaluated price, the contract will be recommended for award on the basis of best value. The following criteria weighted as considered appropriate by the Contracting Authority will be used to determine the best value:

(a) a bidder with an overall satisfactory performance record is given preference over a bidder known to have a less satisfactory performance record;
(b) a bidder in a position to provide adequate after-sales service, with a good record in this regard, will be given preference over a bidder who is less able to provide adequate service or who has a poor record;
(c) when delivery is an important factor, the bidder offering the best delivery date should be given preference;
(d) when there are several items included in the bid and only some items are priced identically, the bid offering the greatest dollar value should be given preference; and
(e) when there are several items included in the bid and one or more bidders bid lower on one or more of the items, the lowest bidder with the greatest dollar value should be given preference both for the items on which it bid equal prices and for the items on which it bid lower.

Canada intends to award up to fifteen (15) contracts to ensure laboratory services are available for all Surveys as identified in Annex A, Statement of Work, however only one contract per Survey will be issued based on the results of the financial evaluation. If a Bidder is recommended for award of a Contract for more than one Survey, the Contract issued will cover all the applicable Surveys to the Bidder.

2.1.3 For Additional Surveys Services: (not a mandatory requirement)

The evaluated price for Additional Surveys Services is determined on the basis of item 6. Additional Surveys in Attachment 1 to Part 3, Financial Bid Presentation Sheet. Responsive bids will be ranked in ascending order of evaluated prices and this ranking will be included in the contracts to be awarded in accordance with 2.1.2. 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 1.4 of Part 7 - Resulting Contract Clauses.

2.1.4 For Additional Analytical Testing Services:

The evaluated price for Additional Analytical Testing Services is determined on the basis of item 7. Additional Analytical Testing Services in Attachment 1 to Part 3, Financial Bid Presentation Sheet. . Responsive bids will be ranked in ascending order of evaluated prices and this ranking will be included in the contracts to be awarded in accordance with 2.1.2. 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.

PART 5 - CERTIFICATIONS

Bidders must provide the required certifications and related documentation to be awarded a contract. Canada will declare a bid non-responsive if the required certifications and related documentation are not completed and submitted as requested.

Compliance with the certifications bidders provide to Canada is subject to verification by Canada during the bid evaluation period (before award of a contract) and after award of a contract. The Contracting Authority will have the right to ask for additional information to verify the bidders' compliance with the certifications before award of a contract. The bid will be declared non-responsive if any certification made by the Bidder is untrue, whether made knowingly or unknowingly. Failure to comply with the certifications to provide the related documentation or to comply with the request of the Contracting Authority for additional information will also render the bid non-responsive.

1. Mandatory Certifications Required Precedent to Contract Award

1.1 Code of Conduct and Certifications - Related documentation

1.1.1 By submitting a bid, the Bidder certifies, for himself and his affiliates, to be in compliance with the Code of Conduct and Certifications clause of the Standard instructions. The related documentation hereinafter mentioned will help Canada in confirming that the certifications are true. By submitting a bid, the Bidder certifies that it is aware, and that its affiliates are aware, that Canada may request additional information, certifications, consent forms and other evidentiary elements proving identity or eligibility. Canada may also verify the information provided by the Bidder, including the information relating to the acts or convictions specified herein, through Independent research, use of any government resources or by contacting third parties. Canada will declare non-responsive any bid in respect of which the information requested is missing or inaccurate, or in respect of which the information contained in the certifications is found to be untrue, in any respect, by Canada. The Bidder and any of the Bidder's affiliates, will also be required to remain free and clear of any acts or convictions specified herein during the period of any contract arising from this bid solicitation.

Bidders who are incorporated, including those bidding as a joint venture, must provide with their bid or promptly thereafter a complete list of names of all individuals who are currently directors of the Bidder. Bidders bidding as sole proprietorship, including those bidding as a joint venture, must provide the name of the owner with their bid or promptly thereafter. Bidders bidding as societies, firms, partnerships or associations of persons do not need to provide lists of names. If the required names have not been received by the time the evaluation of bids is completed, Canada will inform the Bidder of a time frame within which to provide the information. Failure to comply will render the bid non-responsive. Providing the required names is a mandatory requirement for contract award.

Canada may, at any time, request that a Bidder provide properly completed and Signed Consent Forms (Consent to a Criminal Record Verification form- PWGSC-TPSGC 229) (http://www.tpsgc-pwgsc.gc.ca/app-acq/forms/formulaires-forms-eng.html) for any or all individuals aforementioned within the time specified. Failure to provide such Consent Forms within the time period provided will result in the bid being declared non-responsive.

2. Additional Certifications Precedent to Contract Award

The certifications in Attachment 1 to Part 5, Certifications Precedent to Contract Award, should be completed and submitted with the bid but may be submitted afterwards. If any of these required certifications is not completed and submitted as requested, the Contracting Authority will so inform the Bidder and provide the Bidder with a time frame within which to meet the requirement. Failure to comply

with the request of the Contracting Authority and meet the requirement within that time period will render the bid non-responsive.

PART 6 - INSURANCE REQUIREMENTS

1. Insurance Requirements

The Bidder must provide a letter from an insurance broker or an insurance company licensed to operate in Canada stating that the Bidder, if awarded a contract as a result of the bid solicitation, can be insured in accordance with the Insurance Requirements specified in Annex C.

If the information is not provided in the bid, the Contracting Authority will so inform the Bidder and provide the Bidder with a time frame within which to meet the requirement. Failure to comply with the request of the Contracting Authority and meet the requirement within that time period will render the bid non-responsive.

PART 7 - RESULTING CONTRACT CLAUSES

The following clauses and conditions apply to and form part of any contract resulting from the bid solicitation.

1. Statement of Work

The Contractor must perform the Work in accordance with the Statement of Work at Annex A.

1.1 Task Authorization

(a) A portion of the Work, detailed at Annex A, article 11.4, to be performed under the Contract will be on an "as and when requested basis" using a Task Authorization (TA). The Work described in the TA must be in accordance with the scope of the Contract.

(b) An obligation for any Work will come into force only when a Task Authorization (TA) is approved and issued in accordance with the clause entitled “Task Authorization Process”.

(c) As more than one contract has been awarded for the total requirement, a request to perform a task for either article 11.4.3, Additional Surveys Services or article 11.4.4, Additional Analytical Testing Services of the Statement of Work, will be sent to the to the first ranked contractor for the respective services. If that contractor for the respective services confirms in writing that it is unable to perform the task as a result of previous commitments under a TA, the request to perform a task will then be forwarded to the contractor ranked second. This process will continue until the task can be performed by another contractor. If no contractor can perform the task, Canada reserves the right to acquire the required Work by other means. A contractor may advise the Technical Authority and the Contracting Authority in writing that it is unable to carry out additional tasks as a result of previous commitments under a TA and no request to perform a task will be sent to that contractor until that contractor has given notice in writing to the Technical Authority and the Contracting Authority that it is available to perform additional tasks.

1.2 Task Authorization Process

(a) The Technical Authority will provide the Contractor with a description of the task using the Task Authorization form specified in Annex D.

(b) The TA will contain the details of the activities to be performed, a description of the deliverables, and a schedule indicating completion dates for the major activities or submission dates for the deliverables. The TA will also include the applicable basis (bases) and methods of payment as specified in the Contract.

(c) The Contractor must provide the Technical Authority, within ten (10) calendar days of its receipt, the proposed total estimated cost for performing the task and a breakdown of that cost, established in accordance with the Basis of Payment specified in the Contract.

(d) The Contractor must not commence work until a TA authorized by the Technical Authority has been received by the Contractor. The Contractor acknowledges that any work performed before a TA has been received will be done at the Contractor's own risk.

1.3 Task Authorization Limit

The Technical Authority may authorize individual task authorizations up to a limit of $100,000.00, Goods and Services Tax or Harmonized Sales Tax included, inclusive of any revisions.

Any task authorization to be issued in excess of that limit must be recommended by the Technical Authority and authorized by the Contracting Authority before issuance.

1.4 Task Authorization - Order of Ranking for Additional Surveys

___ (*number to be inserted at contract award*) contracts were awarded as a result of Public Works and Government Services Canada (PWGSC) bid solicitation number: 054sq.39903-130313.

The contractors' order of ranking in accordance with pricing for article 11.4.3 of Annex A of the Contract is as follows:

Ranked first (*contractor with the lowest rate for Additional Surveys Services*):_____

Ranked second (*contractor with the second lowest rate for Additional Surveys Services*): _____

(as many lines are to be inserted as there are contractors)

1.5 Task Authorization - Order of Ranking for Additional Analytical Testing Services

___ (*number to be inserted at contract award*) contracts were awarded as a result of Public Works and Government Services Canada (PWGSC) bid solicitation number: 054sq.39903-130313.

The contractors' order of ranking in accordance with pricing for article 11.4.4 of Annex A of the Contract is as follows:

Ranked first (*contractor with the lowest rate for Analytical Testing Services*):_____

Ranked second (*contractor with the second lowest rate for Analytical Testing Services*): _____

(as many lines are to be inserted as there are contractors)

1.6 Canada's Obligation - Portion of the Work - Task Authorizations

Canada's obligation with respect to the portion of the Work under the Contract that is performed through task authorizations is limited to the total amount of the actual tasks performed by the Contractor.

1.7 Periodic Usage Reports - Contracts with Task Authorizations

The Contractor must compile and maintain records on its provision of services to the federal government under authorized Task Authorizations issued under the Contract.

The Contractor must provide this data in accordance with the reporting requirements detailed below. If some data is not available, the reason must be indicated. If services are not provided during a given period, the Contractor must still provide a "NIL" report.

The data must be submitted on a quarterly basis to the Contracting Authority.

The quarterly periods are defined as follows:

1st quarter: April 1 to June 30;
2nd quarter: July 1 to September 30;
3rd quarter: October 1 to December 31; and
4th quarter: January 1 to March 31.

The data must be submitted to the Contracting Authority no later than 15 calendar days after the end of the reporting period.

Reporting Requirement- Details

A detailed and current record of all authorized tasks must be kept for each contract with a task authorization process. This record must contain:

For each authorized task:
(i) the authorized task number or task revision number(s);
(ii) a title or a brief description of each authorized task;
(iii) the total estimated cost specified in the authorized Task Authorization (TA) of each task, GST or HST extra;
(iv) the total amount, GST or HST extra, expended to date against each authorized task;
(v) the start and completion date for each authorized task; and
(vi) the active status of each authorized task, as applicable.

For all authorized tasks:

(i) the amount (GST or HST extra) specified in the contract as Canada's total liability to the contractor for all authorized TAs; and
(ii) the total amount, GST or HST extra, expended to date against all authorized TAs.

2. Standard Clauses and Conditions

All clauses and conditions identified in the Contract by number, date and title are set out in the *Standard Acquisition Clauses and Conditions* Manual (https://buyandsell.gc.ca/policy-and-guidelines/standard-acquisition-clauses-and-conditions-manual) issued by Public Works and Government Services Canada.

2.1 General Conditions

2035 (2012-11-19), General Conditions - Higher Complexity - Services, apply to and form part of the Contract.

3. Term of Contract

3.1 Period of Contract

The period of the Contract is from May 1, 2013 to April 30, 2016.

3.2 Option to Extend the Contract

The Contractor grants to Canada the irrevocable option to extend the term of the Contract by up to two (2) additional one (1) year period(s) under the same conditions. The Contractor agrees that, during the extended period of the Contract, it will be paid in accordance with the applicable provisions as set out in the Basis of Payment.

Canada may exercise this option at any time by sending a written notice to the Contractor at least thirty (30) calendar days prior to the Contract expiry date. The option may only be exercised by the Contracting Authority, and will be evidenced for administrative purposes only, through a contract amendment.

4. Authorities

4.1 Contracting Authority

The Contracting Authority for the Contract is:

Gaëtane Dagenais
Manager, Supply
Public Works and Government Services Canada
Acquisitions Branch
Science Procurement Directorate
Place du Portage, Phase III, 11C1
11 Laurier Street
Gatineau, Quebec
K1A 0S5

Telephone: 819-956-1365
E-mail address: gaetane.dagenais@tpsgc-pwgsc.gc.ca

The Contracting Authority is responsible for the management of the Contract and any changes to the Contract must be authorized in writing by the Contracting Authority. The Contractor must not perform work in excess of or outside the scope of the Contract based on verbal or written requests or instructions from anybody other than the Contracting Authority.

4.2 Technical Authority

The Technical Authority for the Contract is:

Name : (to be identified in the Contract)
Title : _____
Organization : _____
Address : _____

Telephone: ___ ___ _____
Facsimile: ___ ___ _____
E-mail address: _____

The Technical Authority is the representative of the department or agency for whom the Work is being carried out under the Contract and is responsible for all matters concerning the technical content of the Work under the Contract. Technical matters may be discussed with the Technical Authority; however, the Technical Authority has no authority to authorize changes to the scope of the Work. Changes to the scope of the Work can only be made through a contract amendment issued by the Contracting Authority.

4.3 Contractor's Representative

(to be identified in the Contract)

5. Payment

5.1 Basis of Payment

The Contractor will be reimbursed for the costs reasonably and properly incurred in the performance of the Work, as determined in accordance with the Basis of Payment in Annex B, to a limitation of expenditure of $_____ **(***amount to be inserted at contract award*). Customs duties are excluded and Goods and Services Tax or Harmonized Sales Tax is extra, if applicable.

5.1.1 Basis of Payment - Limitation of Expenditure - Task Authorizations

The following Basis of Payment will form part of the approved Task Authorization (TA). The task price will be determined in accordance with the Basis of Payment at Annex B and as follows:

The Contractor will be reimbursed for the costs reasonably and properly incurred in the performance of the Work specified in the authorized Task Authorization (TA), as determined in accordance with the Basis of Payment in Annex B, to the limitation of expenditure specified in the authorized TA.

Canada's liability to the Contractor under the authorized TA must not exceed the limitation of expenditure specified in the authorized TA. Customs duties are _____ (*insert "included", "excluded" OR "subject to exemption"*) and Goods and Services Tax or Harmonized Sales Tax is extra, if applicable.

No increase in the liability of Canada or in the price of the Work specified in the authorized TA resulting from any design changes, modifications or interpretations of the Work will be authorized or paid to the Contractor unless these design changes, modifications or interpretations have been authorized, in writing, by the Contracting Authority before their incorporation into the Work.

5.2 Limitation of Expenditure

1. Canada's total liability to the Contractor under the Contract must not exceed $ _____(*amount to be inserted at contract award*). Customs duties are excluded and Goods and Services Tax or Harmonized Sales Tax is extra, if applicable.

2. No increase in the total liability of Canada or in the price of the Work resulting from any design changes, modifications or interpretations of the Work, will be authorized or paid to the Contractor unless these design changes, modifications or interpretations have been approved, in writing, by the Contracting Authority before their incorporation into the Work. The Contractor must not perform any work or provide any service that would result in Canada's total liability being exceeded before obtaining the written approval of the Contracting Authority. The Contractor must notify the Contracting Authority in writing as to the adequacy of this sum:

(a) when it is 75 percent committed, or

(b) four (4) months before the Contract expiry date, or

(c) as soon as the Contractor considers that the contract funds provided are inadequate for the completion of the Work,

whichever comes first.

(c) If the notification is for inadequate contract funds, the Contractor must provide to the Contracting Authority a written estimate for the additional funds required. Provision of such information by the Contractor does not increase Canada's liability.

5.2.1 Limitation of Expenditure - Cumulative Total of all Task Authorizations

1. Canada's total liability to the Contractor under the Contract for all authorized Task Authorizations (TAs), inclusive of any revisions, must not exceed the sum of $ _____. Customs duties are included and the Goods and Services Tax or Harmonized Sales Tax is extra, if applicable.

2. No increase in the total liability of Canada will be authorized or paid to the Contractor unless an increase has been approved, in writing, by the Contracting Authority.

3. The Contractor must notify the Contracting Authority in writing as to the adequacy of this sum:

(a) when it is 75 percent committed, or
(b) four (4) months before the contract expiry date, or
(c) as soon as the Contractor considers that the sum is inadequate for the completion of the Work required in all authorized TAs, inclusive of any revisions,

whichever comes first.

4. If the notification is for inadequate contract funds, the Contractor must provide to the Contracting Authority, a written estimate for the additional funds required. Provision of such information by the Contractor does not increase Canada's liability.

5.3 Method of Payment

5.3.1 Monthly Payment

Canada will pay the Contractor on a monthly basis for work performed during the month covered by the invoice in accordance with the payment provisions of the Contract if:

(a) an accurate and complete invoice and any other documents required by the Contract have been submitted in accordance with the invoicing instructions provided in the Contract;

(b) all such documents have been verified by Canada;

(c) the Work performed has been accepted by Canada.

5.3.2 Monthly Payment - Approved TA

The following method of payment clause will form part of the approved TA:

For the work specified in an approved TA subject to a Limitation of Expenditure, Canada will pay the Contractor on a monthly basis for work performed during the month covered by the invoice in accordance with the payment provisions of the Task Authorization and the Contract if:

(a) an accurate and complete invoice and any other documents required by the Task Authorization and the Contract have been submitted in accordance with the invoicing instructions provided in the Contract;

(b) all such documents have been verified by Canada;

(c) the Work performed has been accepted by Canada.

5.4 SACC Manual Clauses

A9117C (2007-11-30), T1204 - Direct Request by Customer Department

C0305C (2008-05-12), Cost Submission

5.5 Sample and Time Verification

Testing of samples charged, time charged for laboratory services and the accuracy of the Contractor’s sample recording system and time recording system are subject to verification by Canada, before or after payment is made to the Contractor. If verification is done after payment, the Contractor must repay any overpayment, at Canada’s request.

6. Invoicing Instructions

6.1. The Contractor must submit invoices in accordance with the section entitled “Invoice Submission” of the general conditions. Invoices cannot be submitted until all work identified in the invoice is completed. Each invoice must show the following:

A) Testing

Each invoice must show:

(a) a copy of the sample verification to support the testing surveys claimed;

(b) a copy of the sample submission forms and any other documents as specified in the Contract;

(c) a copy of time sheets to support the time claimed, if applicable.

B) Expert Witness

Each invoice must be supported by:

(a) a list of all expenses, in accordance with the TA;
(b) a copy of time sheets to support the time claimed;
(c) a copy of the release document and any other document(s) as specified in the Contract;
(d) a copy of the invoices, receipts, vouchers for all direct expenses, travel and living expenses.

6.2. Invoices must be distributed as follows:

(a) One (1) copy must be submitted in an electronic format to the Technical Authority identified under the section entitled "Authorities" of the Contract for certification and payment. Microsoft Word, Adobe Reader (.pdf) formats are acceptable.

(b) One (1) copy must be submitted in an electronic format to the Contracting Authority identified under the section entitled “Authorities” of the Contract. Microsoft Word, Adobe Reader (.pdf) formats are acceptable.

7. Certifications

7.1 Compliance with the certifications and related documentation provided by the Contractor in its bid is a condition of the Contract and subject to verification by Canada during the entire contract period. If the Contractor does not comply with any certification, provide the related documentation or if it is determined that any certification made by the Contractor in its bid is untrue, whether made knowingly or unknowingly, Canada has the right, pursuant to the default provision of the Contract, to terminate the Contract for default.

7.2 SACC Manual Clauses

A3060C (2008-05-12), Canadian Content Certification

8. Applicable Laws

The Contract must be interpreted and governed, and the relations between the parties determined, by the laws in force in Ontario.

9. Priority of Documents

If there is a discrepancy between the wording of any documents that appear on the list, the wording of the document that first appears on the list has priority over the wording of any document that subsequently appears on the list.

(a) the Articles of Agreement;
(b) the general conditions 2035 (2012-11-19) General Conditions - Higher Complexity -Services;
(c) Annex A, Statement of Work;
(d) Annex B, Basis of Payment;
(e) Annex C, Insurance requirements;
(f) the signed Task Authorizations
(g) the Contractor's bid dated _____ (*insert date of bid*)

10. Foreign Nationals (Canadian Contractor)

SACC Manual clause A2000C (2006-06-16), Foreign Nationals (Canadian Contractor)

11. Insurance Requirements

The Contractor must comply with the insurance requirements specified in Annex C. The Contractor must maintain the required insurance coverage for the duration of the Contract. Compliance with the insurance requirements does not release the Contractor from or reduce its liability under the Contract.

The Contractor is responsible for deciding if additional insurance coverage is necessary to fulfil its obligation under the Contract and to ensure compliance with any applicable law. Any additional insurance coverage is at the Contractor's expense, and for its own benefit and protection.

The Contractor must forward to the Contracting Authority within ten (10) days after the date of award of the Contract, a Certificate of Insurance evidencing the insurance coverage and confirming that the insurance policy complying with the requirements is in force. Coverage must be placed with an Insurer licensed to carry out business in Canada. The Contractor must, if requested by the Contracting Authority, forward to Canada a certified true copy of all applicable insurance policies.

ATTACHMENT 1 TO PART 3
FINANCIAL BID PRESENTATION SHEET

The Bidder must complete the Financial Bid Presentation Sheet as provided below and include it in its financial bid once completed.

1. <u>Contract period (01-05-2013 to 30-04-2016):</u>

For Sample collection and Analytical testing described under articles 6, 7, 11.2, 11.3 and Tables 1 and 2 of the Statement of Work at Annex A:

The firm all-inclusive unit prices per Survey specified below, when quoted by the Bidder, include any costs associated with, but not limited to, Sample collection, Shipping and handling, Analytical testing, Deliverables, Photos, Reports including adhoc reports, and with Confirmation Procedures, as appropriate**.**

****The estimates for the number of samples provided below are for evaluation purposes only.***

	CONTRACT PERIOD									
	01-05-2013 to 30-04-2014			**01-05-2014 to 30-04-2015**			**01-05-2015 to 30-04-2016**			
Surveys	**Est. # samples ***	**Firm all-inclusive unit price per Survey**	**Subtotal (axb=c)**	**Est. # samples***	**Firm all-inclusive unit price per Survey**	**Sub-total (dxe=f)**	**Est. # of samples***	**Firm all-inclusive unit price per Survey**	**Sub-total (gxh=i)**	**Total per suvey= (c+f+i = j**
	(a)	**(b) ($)**	**(c) ($)**	**(d)**	**(e) ($)**	**(f) ($)**	**(g)**	**(h) ($)**	**(i) ($)**	**(j) ($)**
Acrylamide in selected foods	750			750				Not applicable		
Aflatoxins in selected foods	1,000			1,000			1,000			
Arsenic Speciation in selected foods	1,000			1,000			1,000			
Coumarin in selected foods	750			750			750			
Food colors in selected foods	1,000			1,000			1,000			
Fumonisins in selected foods	750			750			750			

Furans (including 2-methyl and 3-methyl furan) in heat-treated foods	500			500			500			
Glycoalkaloids in Potatoes	500			500			500			
Multi-Mycotoxin Analysis in selected foods	750			750			750			
PBDEs in selected foods	500			500			500			
Perchlorate in selected foods	500			Not applicable						
PFOS/PFOA in selected foods	500			500			500			
Phthalates in selected foods	750			750			750			
Undeclared multiple Allergens in pre-packaged foods	1,200			1,200			1,200			
Undeclared single Allergen in pre-packaged foods	2,000			2,000			2,000			

2. **<u>For Optional period 1 (01-05-2016 to 30-04-2017):</u>**
For Sample collection and Analytical testing described under articles 6, 7, 11.2, 11.3 and Tables 1 and 2 of the Statement of Work at Annex A:

The firm all-inclusive unit prices per Survey specified below, when quoted by the Bidder, include any costs associated with, but not limited to, Sample Collection, Shipping and handling, Analytical Testing, Deliverables, Photos, Reports including adhoc reports, and with Confirmation Procedures, as appropriate**.**

****The estimates for the number of samples provided below are for evaluation purposes only.***

	Optional Period 1 (01-05-2016 to 30-04-2017)		
Surveys	**Est. # samples ***	**Firm all- inclusive unit price per Survey**	**Total per survey (a x b = c)**
	(a)	**(b) ($)**	**(c) ($)**
Acrylamide in selected foods		Not applicable	
Aflatoxins in selected foods	1,000		
Arsenic Speciation in selected foods	1,000		
Coumarin in selected foods	750		
Food colors in selected foods	1,000		
Fumonisins in selected foods	750		
Furans (including 2-methyl and 3-methyl furan) in heat-treated foods	500		
Glycoalkaloids in Potatoes		Not applicable	
Multi-Mycotoxin Analysis in selected foods	750		
PBDEs in selected foods	500		
Perchlorate in selected foods		Not applicable	
PFOS/PFOA in selected foods	500		
Phthalates in selected foods	750		
Undeclared multiple Allergens in pre-packaged foods	1,200		
Undeclared single Allergen in pre-packaged foods	2,000		

3. **<u>For Optional period 2 (01-05-2017 to 30-04-2018)</u>**
For Sample collection and Analytical testing described under articles 6, 7, 11.2, 11.3 and Tables 1 and 2 of the Statement of Work at Annex A:

The firm all-inclusive unit prices per Survey specified below, when quoted by the Bidder, include any costs associated with, but not limited to, Sample Collection, Shipping and handling, Analytical Testing, Deliverables, Photos, Reports including adhoc reports, and with Confirmation Procedures, as appropriate**.**

****The estimates for the number of samples provided below are for evaluation purposes only.***

	Optional Period 2 (01-05-2017 to 30-04-2018)		
Surveys	**Est. # samples ***	**Firm all- inclusive unit price per Survey**	**Total per survey (a x b=c)**
	(a)	**(b) ($)**	**(c) ($)**
Acrylamide in selected foods		Not applicable	
Aflatoxins in selected foods	1,000		
Arsenic Speciation in selected foods	1,000		
Coumarin in selected foods	750		
Food colors in selected foods	1,000		
Fumonisins in selected foods	750		
Furans (including 2-methyl and 3-methyl furan) in heat-treated foods	500		
Glycoalkaloids in Potatoes		Not applicable	
Multi-Mycotoxin Analysis in selected foods	750		
PBDEs in selected foods	500		
Perchlorate in selected foods		Not applicable	
PFOS/PFOA in selected foods	500		
Phthalates in selected foods	750		
Undeclared multiple Allergens in pre-packaged foods	1,200		

Undeclared single Allergen in pre-packaged foods	2,000		

Task Authorization Portion:

4. <u>For Optional Survey Packages on an "as and when requested" basis</u> as described under article 11.4.1, 11.2 and 11.3 and Table 3 of the Statement of Work at Annex A:

The firm all-inclusive unit prices per Survey specified below, when quoted by the Bidder, include any costs associated with, but not limited to, Sample Collection, Shipping and handling, Analytical Testing, Deliverables, Photos, Reports including adhoc reports, and with Confirmation Procedures, as appropriate**.**

****The estimates for the number of samples provided below are for evaluation purposes only.***

Survey Name	**Estimated Additional Number of samples yearly above the number of samples in Table 1 of SOW***	**Contract Period**						**Optional Period 1**		**Optional Period 2**		
		01-05-2013 to 30-04-2014		**01-05-2014 to 30-04-2015**		**01-05-2015 to 30-04-2016**		**01-05-2016 to 30-04-2017**		**01-05-2017 to 30-04-2018**		
		Firm all-inclusive unit price for Survey	**Sub-total (a x b= c)**	**Firm all-inclusive unit price for Survey**	**Sub-total (a x d= e)**	**Firm all-inclusive unit price per Survey**	**Sub-total (a x f= g)**	**Firm all-inclusive unit price per Survey**	**Sub-total (a x h =i)**	**Firm all-inclusive unit price per Survey**	**Sub-total (a x j=k)**	**Total per Survey (c + e + g + i + k = l)**
	(a)	**(b) ($)**	**(c) ($)**	**(d) ($)**	**(e) ($)**	**(f) ($)**	**(g) ($)**	**(h) ($)**	**(i) ($)**	**(j) ($)**	**(k) ($)**	**(l) ($)**
Aflatoxins in selected foods	1000											
Arsenic Speciation in selected foods	1,000											
Coumarin in selected foods	750											
Food colors in selected foods	1,000											
Fumonisins in selected foods	750											
Furans (including 2-methyl and 3-methyl furan) in heat-treated foods	750											
Multi-Mycotoxin Analysis in selected foods	750											
PBDEs in selected foods	500											

PFOS/PFOA in selected foods	500											
Phthalates in selected foods	750											
Undeclared multiple Allergens in pre-packaged foods	600											
Undeclared single Allergen in pre-packaged foods	1,000											

5. **<u>For Expert Testimony services on an "as and when requested" basis</u> as described under article 11.4.2 of the Statement of Work:**
The firm all-inclusive daily rates, when quoted by the Bidder, are inclusive of overhead and profit.

The estimates provided below are for evaluation purposes only.

Estimated # of days per year *	Contract Period						Optional Period 1		Optional Period 2	
	01-05-2013 to 30-04-2014		01-05-2014 to 30-04-2015		01-05-2015 to 30-04-2016		01-05-2016 to 30-04-2017		01-05-2017 to 30-04-2018	
	Firm all-inclusive daily rate [1]	Sub-total (a x b= c)	Firm all-inclusive daily rate [1]	Sub-total (a x d= e)	Firm all-inclusive daily rate [1]	Sub-total (a x f= g)	Firm all-inclusive daily rate [1]	Sub-total (a x h= i)	Firm all-inclusive daily rate [1]	Sub-total (a x j= k)
(a)	(b) ($)	(c) ($)	(d) ($)	(e) ($)	(f) ($)	(g) ($)	(h) ($)	(i) ($)	(j) ($)	(k) ($)
1										
Total for 5. = (c + e + g + i + k)										

[1]Definition of a Day/Prorating: A day is defined as 7.5 hours exclusive of meal breaks. Payment will be for days actually worked with no provision for annual leave, statutory holidays and sick leave. Time worked (Days worked, in the formula below) which is less than a day will be prorated to reflect actual time worked in accordance with the following formula:

$$\text{Days worked} = \frac{\text{Hours worked}}{\text{7.5 hours per day}}$$

6. **For Additional Surveys services on an "as and when requested" basis in accordance with 11.4.3, 11.2 and 11.3 of Annex A:**
The firm all-inclusive hourly rates specified below, when quoted by the Bidder, include any costs associated with, but not limited to, Sample Collection, shipping and handling, Analytical Testing, Deliverables, Photos, Reports including ad hoc reports and with Confirmation Procedures, as appropriate**.**

****The estimates provided below are for evaluation purposes only.***

Contract Period							Optional Period 1			Optional Period 2		
Estimated # of hours * during the contract period	01-05-2013 to 30-04-2014		01-05-2014 to 30-04-2015		01-05-2015 to 30-04-2016		01-05-2016 to 30-04-2017			01-05-2017 to 30-04-2018		
	Firm all-inclusive hourly rate	Sub-total (a x b= c)	Firm all-inclusive hourly rate	Sub-total (a x d= e)	Firm all-inclusive hourly rate	Sub-total (a x f= g)	Estimated # of hours during Optional Period 1 *	Firm all-inclusive hourly rate	Sub-total (h x i = j)	Estimated # of hours during Optional Period 2 *	Firm all-inclusive hourly rate	Sub-total (k x l = m
(a)	(b) ($)	(c) ($)	(d) ($)	(e) ($)	(f) ($)	(g) ($)	(h)	(i) ($)	(j) ($)	(k)	(l) ($)	(m) ($)
1000							300			300		
Total for 6. = (c+ e + g + j + m)												

7. **For additional Analytical Testing services "on an as and when requested" basis in accordance with 11.4.4, 11.2 and 11.3 of of the Statement of Work at Annex A:**
The firm all-inclusive hourly rates specified below, when quoted by the Bidder, include any costs associated with, but not limited to, Analytical Testing, Deliverables, Photos, Reports including ad hoc reports and with Confirmation Procedures, as appropriate**.**

****The estimates provided below are for evaluation purposes only.***

Contract Period				Optional Period 1	Optional Period 2
	01-05-2013 to 30-04-2014	01-05-2014 to 30-04-2015	01-05-2015 to 30-04-2016	01-05-2016 to 30-04-2017	01-05-2017 to 30-04-2018

Estimated # of hours * during the contract period	Firm all-inclusive hourly rate	Sub-total (a x b= c)	Firm all-inclusive hourly rate	Sub-total (a x d= e)	Firm all-inclusive hourly rate	Sub-total (a x f= g)	Estimated # of hours during Optional Period 1 *	Firm all-inclusive hourly rate	Sub-total (h x i = j)	Estimated # of hours during Optional Period 2 *	Firm all-inclusive hourly rate	Sub-total (k x l = m
(a)	(b) ($)	(c) ($)	(d) ($)	(e) ($)	(f) ($)	(g) ($)	(h)	(i) ($)	(j) ($)	(k)	(l) ($)	(m) ($)
25							25			25		
Total for 7. = (c+ e + g + j + m)												

ATTACHMENT 1 TO PART 4
MANDATORY TECHNICAL CRITERIA

Item	Description	Met	Not Met
M1	For sample collection, the Bidder and (or) its proposed subcontractors must demonstrate that it has a facility in Canada. For laboratory testing, the Bidder must demonstrate that it has a laboratory located in Canada that offers analytical tests accredited by the Standards Council of Canada (SCC) under the Program Specialty Area for Agriculture & Food Products, or accredited by the Canadian Association of Laboratory Accreditation (CALA) in field of testing for Food. A copy of the Certificate of Accreditation from SCC and / or CALA must be provided.		
M2	The Bidder must submit a control copy [1] of their analytical methods with the detailed Standard Operating Procedure (SOP) for the testing of hazards identified in Appendix I to Annex A that meet either the criteria of M2.1 or M2.2: **M2.1** The analytical methods must be accredited by the SCC or CALA for the food samples. To demonstrate accreditation by the SCC or CALA, the Bidder must: **M2.1.1** Identify the SOP by title and SOP # if applicable; OR **M2.1.2** In the event that the method has been accredited by the SCC or CALA but the test accreditation has not yet been posted on its website at the time the bid is submitted, the Bidder must provide a signed letter from the Accreditation body to that effect (see 6.3 in SOW). **Or** **M2.2** In the event the SCC or CALA has not yet accredited the method, the Bidder must provide sufficient validation documentation to demonstrate acceptability according to: CODEX ALIMENTARIUS COMMISSION procedure manual Twentieth edition ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_		

	20e.pdf. Table 1: Guidelines for establishing numeric values for the criteria, on page 66.		
M3	For allergen testing, the Bidder must demonstrate its ability to meet the Limit of Detection (LOD) published by the supplier of the testing kit, as described in Table 5. **M3.1** Matrix extension or full validation records must be supplied to demonstrate valid detection limits in flavor packets, cooking sauces, Chicken soup broth matrix for the Allergen kits.		
M4	The Bidder must submit analytical methods that meet the mandatory requirements for LOD and Limit of Quantitation (LOQ) as identified in Appendix I to Annex A, Reference Methods and Criteria. To demonstrate compliance, the control copy[1] of the SOP submitted for a particular test must clearly indicate these mandatory requirements are met; this includes clearly stating the LOD and LOQ in Table 5, Technical Evaluation Table for all the analytes determined by the analytical method, in accordance to the specific sample matrix, preferably the recommended matrix. Add extra row(s) where more than one matrix has been validated for the methods.		
M5	The Bidder must have the capacity to carry out the analysis on the Hazards it is bidding on. To demonstrate the capacity, the Bidder must provide the information on Instrumentation and weekly throughput using **Table 5** Technical Evaluation Table for the Hazard(s) it is bidding on.		
M6	The Bidder and (or) its proposed subcontractors must demonstrate the ability to provide the sample collection service from all six (6) metropolitan areas, identified in article 7.1.2. in Annex A, Statement of work. To demonstrate, the Bidder and (or) its proposed subcontractors must provide: **M6.1** A control copy [1] of SOPs including sample collection, handling, shipping, receiving and sample verification in accordance with Appendix II, IV and V to Annex A, Statement of Work.		

M6.2 The physical addresses and photos, of the storage facilities before shipping in each metropolitan area.

City	Address [2] and location [2]
Calgary	
Halifax	
Montreal	
Ottawa	
Toronto	
Vancouver	

M7 The Bidder must demonstrate that it is capable of providing the proper storage for samples upon receipt at the Laboratory as follows:

M7.1 The Bidder must have temperature monitoring system implemented to these storage facilities in accordance with Appendix V to Annex A, Sample Storage and Shipping Criteria. To demonstrate, the bidder must provide a copy of recent temperature records of these laboratory storage facilities.

M7.2 The Bidder must demonstrate that these laboratory storage facilities are capable of accommodating the volume of the sample material as estimated in Table 1, Survey Sample Guidance in Annex A, Statement of work. To demonstrate, the bidder must provide location, photos of these storages and following information.

	Ambient Storage	Refrigerator (s)/ Walk in fridge	Freezer(s)
Location [2] in Detail			
Capacity [3] (in $ft^{3)}$			

1. Control Copy is a copy of SOP signed and dated by QA personnel.
2. Address and Location must be provided in detail, i.e. street address and room number. If there are more than one (1) locations in a city, please list all.
3. All capacities are measured in ft^3 and must be greater than 0. A minimum of 100 ft^3 of total capacity within one city is required.

Table 5 Technical Evaluation Table (Method Parameters)

Hazard	Recommended Matrix for Evaluation	Reference Standard used[1]	Applicable Analytes	LOD[2] / LOQ[2]	Calibration Range	Primary and Secondary Instrumentation[3]	Currently Validated Matrices	Scheduled Matrices requiring Validation	Expected Weekly Throughput
Example Hazard	Cookies	NIST RSM 2387	Analyte A Analyte B	1 / 5 ppb 2 / 6 ppb	2.5 – 20 ppb 2.5 – 20 ppb	LC-MS/MS / GC-ECD	Pudding, Jello, Cookies	Sugar Candies	2 set x 20 spls
Acrylamide	Olives		Acrylamide						
Aflatoxins	Pistachios		Aflatoxin B1						
			Aflatoxin B2						
			Aflatoxin G1						
			Aflatoxin G2						
Arsenic Speciation	Seaweed		AS^{3+}						
			DMA						
			MMA						
			AS^{5+}						
Coumarin	Breakfast cereal with no cinamon content		coumarin						
Food Colours	Energy Sport Drink (ex: Redbull)		Sunset Yellow FCF						
			Amaranth						
			Infigo Carmine						
			Fast Green FCG						
			Brilliant Blue FCF						
			Allura Red						
			Ponceau SX						
			Erythrosin B, Ponceau 4R						
			Azorubine						

Hazard	Recommended Matrix for Evaluation	Reference Standard used[1]	Applicable Analytes	LOD[2] / LOQ[2]	Calibration Range	Primary and Secondary Instrumentation[3]	Currently Validated Matrices	Scheduled Matrices requiring Validation	Expected Weekly Throughput
			Lissamine Green						
			Patent Blue Violet						
			Rhodamine B						
Fumonisins	Quinoa or Barley based cereal		Fumonisin B1						
			Funomisin B2						
Furans	Pretzels		2-methyl Furan						
			3-methyl Furan						
Glycoalkaloids	Fresh Potato tubers		Solanine						
			Chaconine						
Multi-Mycotoxin analysis	Grain based crackers		AFB1						
			AFB2						
			AFG1						
			AFG2						
			STE						
			CPA						
			OTA						
			DON						
			NIV						
			FUS-X						
			3-AcDON						
			15-AcDON						
			NEO						
			DAS						
			HT-2						
			T-2						
			FB1						
			FB2						
			FB3						

Hazard	Recommended Matrix for Evaluation	Reference Standard used[1]	Applicable Analytes	LOD[2] / LOQ[2]	Calibration Range	Primary and Secondary Instrumentation[3]	Currently Validated Matrices	Scheduled Matrices requiring Validation	Expected Weekly Throughput
			ZEA						
			α-ZOL						
			β-ZOL						
			ergocristine						
			ergocryptine						
			ergosine						
PBDEs	Nut butter		BDE-17,						
			BDE-28						
			BDE-47						
			BDE-66						
			BDE-77						
			BDE-85						
			BDE-99						
			BDE-100						
			BDE-138						
			BDE-153						
			BDE-154						
			BDE-183						
			BDE-209						
Perchlorate	Cantaloupe		Perchlorate						
PFOS/PFOA	Microwave Pop Corn		PFOS						
			PFOA						
Phthalates	Infant Formula		BBP						
			DBP						
			DEHP						
			DNOP						
			DINP						
			DIDP						
Allergens	Snack food (mixed nuts) with no Almond declared		Almond						

Hazard	Recommended Matrix for Evaluation	Reference Standard used[1]	Applicable Analytes	LOD[2] / LOQ[2]	Calibration Range	Primary and Secondary Instrumentation[3]	Currently Validated Matrices	Scheduled Matrices requiring Validation	Expected Weekly Throughput
	Crackers with no milk content declared		BLG						
	Crackers with no milk content declared		Casein						
	Granola Bar with no egg content declared		Egg						
	Gluten free crackers		Gluten						
	Granola Bar with no Hazelnut declared		Hazelnut						
	Sauces with no mustard declared		Mustard						
	Snack food (with almonds) with no peanut declared		Peanut						
	Bagels with no sesame declared		Sesame						
	Sauces with no soy declared		Soy						

1. A Certificate of Analysis of the Reference Material must be provided. In case of certified Reference Material is not commercially available, provide the details of the material used.
2. To demonstrate LOD/LOQ criteria can be achieved by submitted method, the Bidder must provide a chromatogram (or readings in the case of ELISA) fortified at the LOD and LOQ in the specified matrix as well as a representative blank matrix.
3. Provide alternative testing kit information in case of Allergen testing methods.

ATTACHMENT 2 TO PART 4
EVALUATION OF PRICE

The financial bids submitted in response to this solicitation will be assessed in accordance with sections A, B and C below:

Where no firm unit prices are required, $0.00 will be used for evaluation purposes.

A. The evaluated price for each Survey will be calculated as follows:

1: Contract Period;

(a) 01-05-2013 to 30-04-2014
Subtotal = multiplying the firm all-inclusive unit prices per Survey by the estimated number of samples stipulated in the Financial Bid Presentation Sheet
Total for each Survey in 1(a) = Sum of the Subtotal per Survey

(b) 01-05-2014 to 30-04-2015
Subtotal = multiplying the firm all-inclusive unit prices per Survey by the estimated number of samples stipulated in the Financial Bid Presentation Sheet
Total for each Survey in 1(b) = Sum of the Subtotal per Survey

(c) 01-05-2015 to 30-04-2016
Subtotal = multiplying the firm all-inclusive unit prices per Survey by the estimated number of samples stipulated in the Financial Bid Presentation Sheet
Total for each Survey in 1(c) = Sum of the Subtotal per Survey

Total for 1 = Sum of total for 1(a) + Sum of total for 1(b) + Sum of Total for 1(c)

2. Optional Period 1 (01-05-2016 to 30-04-2017)
Subtotal = multiplying the firm all-inclusive unit prices per Survey by the estimated number of samples stipulated in the Financial Bid Presentation Sheet
Total for 2. = Sum of the Subtotal per Survey for Optional Period 1.

3. Optional Period 2 (01-05-2017 to 30-04-2018)
Subtotal = multiplying the firm all-inclusive unit price per Survey by the estimated number of samples stipulated in the Financial Bid Presentation Sheet
Total for 3. = **Sum of the Subtotal per Survey for Optional Period 2.**

4. Optional Survey Packages:

Subtotal = multiplying the firm all-inclusive unit price per Survey for each of the 3 contract periods and each of the optional periods, by the maximum number of samples stipulated in the Financial Bid Presentation Sheet for each period
Total of 4. = **Sum of the Subtotal per Survey for the contract period and each of the 2 optional periods**

5. For Expert Testimony services:

Subtotals: multiplying the firm all inclusive daily rate for the contract period and each of the optional periods, by the estimated number of days stipulated in the Financial Bid Presentation Sheet
Total of 5. = **Sum of the Subtotal for Expert Testimony for the contract period and each of the 2 optional periods**

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
(GST/HST EXCLUDED)

Example A: Bidder A

Bidder A submitted a bid the evaluated price for Acrylamide in selected foods and Expert Testimony is calculated as follows:

1: For Contract Period (01-05-2013 to 30-04-2016):

	CONTRACT PERIOD									
	01-05-2013 to 30-04-2014			01-05-2014 to 30-04-2015			01-05-2015 to 30-04-2016			
Surveys	Est. # samples *	Firm all-inclusive unit price per Survey	Subtotal (axb=c)	Est. # samples*	Firm all-inclusive unit price per Survey	Sub-total (dxe=f)	Est. # of samples*	Firm all-inclusive unit price per Survey	Sub-total (gxh=i)	Total per suvey= (c+f+i = j
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
Acrylamide in selected foods	750	$40.00	750 x $40.00 = $30,000.00	750	$41.00	750 x $41.00 = $30,750.00		Not applicable		$60,750.00

Total for Acrylamide in selected foods for 1. = $60,750.00

2. For Optional Period 1:

Acrylamide in selected foods: Not required

No firm unit prices are required, $0.00 is used.

3. For Optional Period 2:

Acrylamide in selected foods: Not required

No firm unit prices are required, $0.00 is used.

4. For Optional Survey Packages:

Acrylamide in selected foods: Not required

No firm unit prices are required, $0.00 is used.

5. For Expert Testimony services (for each Survey):

Estimated # of days per year	Contract Period						Optional Period 1		Optional Period 2	
	01-05-2013 to 30-04-2014		01-05-2014 to 30-04-2015		01-05-2015 to 30-04-2016		01-05-2016 to 30-04-2017		01-05-2017 to 30-04-2018	
	Firm all-inclusive daily rate	Sub-total (a x b= c)	Firm all-inclusive daily rate	Sub-total (a x d= e)	Firm all-inclusive daily rate	Sub-total (a x f= g)	Firm all-inclusive daily rate	Sub-total (a x h= i)	Firm all-inclusive daily rate	Sub-total (a x j= k)
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
1	$325.00	1 x 325.00 = $325.00	$330.00	1 x 330.00 = $330.00	$335.00	1 x 335.00 = $335.00	$340.00	1 x 340.00 = $340.00	$345.00	1 x 345.00 = $345.00
Total = (c + e + g + i + k) = $325.00 + $330.00 + $335.00 + $340.00 = $345.00 = $1340.00										

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

Selection will be in accordance with article 2. Basis of Selection, Part 4 – Evaluation Procedures and Basis of Selection in the bid solicitation.

B. The evaluated price for Additional Surveys services will be calculated as follows:

1. Additional Surveys:
Subtotals: multiplying the firm all inclusive hour rate for each of the 3 years in the contract period and each of the 2 optional periods, by the estimated number of days stipulated in the Financial Presentation Sheet
Total evaluated price for B. = Sum of the Subtotals for Additional Surveys for the 3 years in the contract period and each of the 2 optional periods

GST/HST EXCLUDED

Example B.: Bidder A

As Bidder A submitted a bid for 6. Additional Surveys services, the evaluated price will be calculated as follows:

Contract Period							Optional Period 1			Optional Period 2		
Estimated # of hours	01-05-2013 to 30-04-2014		01-05-2014 to 30-04-2015		01-05-2015 to 30-04-2016		01-05-2016 to 30-04-2017			01-05-2017 to 30-04-2018		
	Firm all-inclusive hourly rate	Sub-total (a x b= c)	Firm all-inclusive hourly rate	Sub-total (a x d= e)	Firm all-inclusive hourly rate	Sub-total (a x f= g)	Estimated # of hours	Firm all-inclusive hourly rate	Sub-total (h x i = j)	Estimated # of hours	Firm all-inclusive hourly rate	Sub-total (k x l = m
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)	(l)	(m)
1000	$40.00	1000 x $40.00 = $40,000.00	$41.00	1000 x $41.00 = $41,000.00	$42.00	1000 x $42.00 = $42,000.00	300	$43.00	300 x $43.00 = $12,900.00	300	$44.00	300 x $44.00 = $13,200.00
Total for B.1. = (c+ e + g + j + m) = $40,000.00 + $41,000.00 + $42,000.00 + $12,900.00 + $13,200.00 = $149,000.00												

The total evaluated price for B.1. Additional Surveys services is $149,000.00

Selection will be in accordance with article 2. Basis of Selection, Part 4 – Evaluation Procedures and Basis of Selection in the bid solicitation.

C. The evaluated price for Additional Analytical Testing services will be calculated as follows:

1. Additional Analytical Testing:
   Subtotals: multiplying the firm all inclusive hour rate for each of the 3 years in the contract period and each of the 2 optional periods, by the estimated number of days stipulated in the Financial Presentation Sheet
   Total evaluated price for C. = Sum of the Subtotals for Additional Surveys for the 3 years in the contract period and each of the 2 optional periods

   GST/HST EXCLUDED

Example C.: Bidder A

Bidder A submitted a bid for C.1. Additional Analytical Testing services and the evaluated price will be calculated as follows:

1. For Additional Analytical Testing services :

Contract Period							Optional Period 1			Optional Period 2		
Estimated # of hours	01-05-2013 to 30-04-2014		01-05-2014 to 30-04-2015		01-05-2015 to 30-04-2016		01-05-2016 to 30-04-2017			01-05-2017 to 30-04-2018		
	Firm all-inclusive hourly rate	Sub-total (a x b= c)	Firm all-inclusive hourly rate	Sub-total (a x d= e)	Firm all-inclusive hourly rate	Sub-total (a x f= g)	Estimated # of hours during Optional Period 1	Firm all-inclusive hourly rate	Sub-total (h x i = j)	Estimated # of hours during Optional Period 2	Firm all-inclusive hourly rate	Sub-total (k x l = m
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)	(l)	(m)

25	$30.00	**25 x $30.00 = $750.00**	$31.00	**25 x $31.00 = $775.00**	$32.00	**25 x $32.00 = $800.00**	25	$33.00	**25 x $33.00 = $825.00**	25	$34.00	**25 x $34.00 = $850.00**
Total for C.1 = (c+ e + g + j + m) = $750.00 +$775.00 + $800.00 + $825.00 + $850.00 = $4,000.00												

The total evaluated price for C.1. Additional Analytical Testing is: $ 4,000.00

Selection will be in accordance with article 2. Basis of Selection, Part 4 – Evaluation Procedures and Basis of Selection in the bid solicitation.

ATTACHMENT 1 to Part 5

CERTIFICATIONS PRECEDENT TO CONTRACT AWARD

1. Federal Contractors Program for Employment Equity - Certification

1.1 Federal Contractors Program - $200,000 or more

1. The Federal Contractors Program (FCP) requires that some suppliers, including a supplier who is a member of a joint venture, bidding for federal government contracts, valued at $200,000 or more (including all applicable taxes), make a formal commitment to implement employment equity. This is a condition precedent to contract award. If the Bidder, or, if the Bidder is a joint venture and if any member of the joint venture, is subject to the FCP, evidence of its commitment must be provided before the award of the Contract.

   Suppliers who have been declared ineligible contractors by Human Resources and Skills Development Canada (HRSDC) are no longer eligible to receive government contracts over the threshold for solicitation of bids as set out in the *Government Contracts Regulations*. Suppliers may be declared ineligible contractors either as a result of a finding of non-compliance by HRSDC, or following their voluntary withdrawal from the FCP for a reason other than the reduction of their workforce to less than 100 employees. Any bids from ineligible contractors, including a bid from a joint venture that has a member who is an ineligible contractor, will be declared non-responsive.

2. If the Bidder does not fall within the exceptions enumerated in 3.(a) or (b) below, or does not have a valid certificate number confirming its adherence to the FCP, the Bidder must fax (819-953-8768) a copy of the signed form LAB 1168, Certificate of Commitment to Implement Employment Equity, to the Labour Branch of HRSDC.

3. The Bidder, or, if the Bidder is a joint venture the member of the joint venture, certifies its status with the FCP, as follows:

   The Bidder or the member of the joint venture

   (a) ( ) is not subject to the FCP, having a workforce of less than 100 full-time or part-time permanent employees, and/or temporary employees having worked 12 weeks or more in Canada;

   (b) ( ) is not subject to the FCP, being a regulated employer under the *Employment Equity Act*, S.C. 1995, c. 44**;**

   (c) ( ) is subject to the requirements of the FCP, having a workforce of 100 or more full-time or part-time permanent employees, and/or temporary employees having worked 12 weeks or more in Canada, but has not previously obtained a certificate number from HRSDC (having not bid on requirements of $200,000 or more), in which case a duly signed certificate of commitment is attached;

   (d) ( ) is subject to the FCP, and has a valid certificate number as follows: _____ (e.g. has not been declared an ineligible contractor by HRSDC.)

   Further information on the FCP is available on the HRSDC Web site (http://www.hrsdc.gc.ca/eng/labour/equality/fcp/index.shtml).

2. Former Public Servant Certification

Contracts with former public servants (FPS) in receipt of a pension or of a lump sum payment must bear the closest public scrutiny, and reflect fairness in the spending of public funds. In order to comply with Treasury Board policies and directives on contracts with FPS, bidders must provide the information required below.

Definitions

For the purposes of this clause,

"former public servant" is any former member of a department as defined in the *Financial Administration Act*, R.S., 1985, c. F-11, a former member of the Canadian Armed Forces or a former member of the Royal Canadian Mounted Police. A former public servant may be:

(a) an individual;
(b) an individual who has incorporated;
(c) a partnership made of former public servants; or
(d) a sole proprietorship or entity where the affected individual has a controlling or major interest in the entity.

"lump sum payment period" means the period measured in weeks of salary, for which payment has been made to facilitate the transition to retirement or to other employment as a result of the implementation of various programs to reduce the size of the Public Service. The lump sum payment period does not include the period of severance pay, which is measured in a like manner.

"pension" means, in the context of the fee abatement formula, a pension or annual allowance paid under the *Public Service Superannuation Act* (PSSA), R.S., 1985, c. P-36, and any increases paid pursuant to the *Supplementary Retirement Benefits Act*, R.S., 1985, c. S-24 as it affects the PSSA. It does not include pensions payable pursuant to the *Canadian Forces Superannuation Act,* R.S., 1985, c. C-17, the *Defence Services Pension Continuation Act*, 1970, c. D-3, the *Royal Canadian Mounted Police Pension Continuation Act*, 1970, c. R-10, and the *Royal Canadian Mounted Police Superannuation Act*, R.S., 1985, c. R-11, the *Members of Parliament Retiring Allowances Act*, R.S., 1985, c. M-5, and that portion of pension payable to the *Canada Pension Plan Act*, R.S., 1985, c. C-8.

Former Public Servant in Receipt of a Pension

Is the Bidder a FPS in receipt of a pension as defined above? **YES ( ) NO ( )**

If so, the Bidder must provide the following information:

(a) name of former public servant;
(b) date of termination of employment or retirement from the Public Service.

Work Force Reduction Program

Is the Bidder a FPS who received a lump sum payment pursuant to the terms of a work force reduction program? **YES ( ) NO ( )**

If so, the Bidder must provide the following information:

(a) name of former public servant;
(b) conditions of the lump sum payment incentive;
(c) date of termination of employment;
(d) amount of lump sum payment;

(e) rate of pay on which lump sum payment is based;
(f) period of lump sum payment including start date, end date and number of weeks;
(g) number and amount (professional fees) of other contracts subject to the restrictions of a work force reduction program.

For all contracts awarded during the lump sum payment period, the total amount of fees that may be paid to a FPS who received a lump sum payment is $5,000, including the Goods and Services Tax or Harmonized Sales Tax.

Certification

By submitting a bid, the Bidder certifies that the information submitted by the Bidder in response to the above requirements is accurate and complete.

3. Canadian Content Certification

This procurement is limited to Canadian services.

The Bidder certifies that:

( ) the service(s) offered is(are) a Canadian service as defined in paragraph 2 of clause A3050T.

3.1 SACC Manual clause A3050T (2010-01-11), Canadian Content Definition

ANNEX A

STATEMENT OF WORK

When the word "shall" is used in the appendices, including attachments, to Annex A, it is to be read and interpreted as "must".

1.0 Title

Analytical services for the detection and quantitation of allergens, chemical additives and residue contaminants in food for the provision of Targeted Survey for the Canadian Food Inspection Agency (CFIA)

2.0 Definitions

Survey	The targeted allergens, chemical additives and residue contaminants of food Survey prescribed by CFIA to evaluate various foods for specific Hazards in the Canadian market. These Surveys map baseline data for certain food products available in Canada at a given date, i.e. sample specifications and testing results.
Hazard	Each Hazard represents a Survey. A Hazard is a source of potential damage, harm or adverse health effect on someone from food.
Analytical Method	Method offered by the Contractor in its Standard Operating Procedure (SOP) for the detection and-or quantitation of allergens, chemical additives and residue contaminants of food of interest to CFIA
Reference Method	A method provided by CFIA, to which the Contractor's method must be approved equivalent.
SOP	The standard operating procedures that the Contractor submitted in its bid for a particular Survey. These procedures may cover laboratory testing, sample handling and other related activities.
LOD (Limit of Detection)	The lowest level of an analyte that may be detected by the submitted SOP in parts per million (ppm), or noted as otherwise
LOQ (Limit of Quantitation)	The limit of an analyte that may be quantitated by the submitted SOP in parts per million (ppm), or noted as otherwise
Food	As defined in *Canadian Food and Drug Act*, food includes any product manufactured, sold or represented for use as food or drink for human beings, chewing gum, and any ingredient that may be mixed with food for any purpose whatever.
Commodity	The types of food identified as dairy, egg, meat, honey, fresh, processed or Imported and Manufactured Food Division (IMFD)
Product Type	Description used by Technical Authority for a group of similar food products. i.e. dried fruit, infant formula – soy.
Working day	Any day between and including Monday to Friday, but not national or provincial (the province where the Contractor's lab is) holidays.

3.0 Statement of Work (SOW) Terminology

3.1 Acronyms

AOAC - Association of Analytical Communities
CALA – Canadian Association for Laboratory Accreditation
ELISA – Enzyme-linked Immunosorbent Assay
ESI - Electrospray Ionization
FAPAS – Food Analysis Performance Assessment Scheme
FCSAP – Food and Consumer Safety Action Plan
FSAP - Food Safety Action Plan

GC – Gas Chromatography
IC – Ion Chromatography
LC – Liquid Chromatography
HPLC – High Performance Liquid Chromatography
MRL – Maximum Residue Limits
MS or MSD – Mass Spectrometry
SCC – Standards Council of Canada
SPE – Solid Phase Extraction
UV – Ultraviolet
ICP – Inductively coupled plasma
IMFD – Imported and Manufactured Food Division

3.2 Forms/Reports

Sample Submission Form	'Form'
Report of Analysis	'RoA'
Monthly Sample Collection Reports	'Report #1'
Monthly Results Reports	'Report #2'

4.0 Objective

The objective of the Work is for the provision of Laboratory services for the delivery of targeted Surveys of allergens, chemical additives and residue contaminants in food for the Canadian Food Inspection Agency (CFIA) in accordance with the identified Hazards that are listed in Appendix I to Annex A, Reference Methods and Criteria.

Analytical testing services must be performed in a laboratory accredited under the Standard Council of Canada (SCC) or the Canadian Association for Laboratory Accreditation (CALA) for the work requirements. Further information on the accreditation process may be found at the following websites:

(a) SCC - http://www.scc.ca/en/about-scc/publications/criteria-and-procedures/laboratory-accreditation

(b) CALA - http://www.cala.ca/accred_program.html

5.0 Background

The CFIA is a federal regulatory agency with a mandate to safeguard food, animals and plants to enhance the health and well-being of Canada's people, environment and economy

In December 2007 the Government of Canada announced the Food and Consumer Safety Action Plan (FCSAP), a comprehensive set of proposed new measures that will make Canadians safer by legislating tougher federal government regulation of food, health, and consumer products. The Food Safety Action Plan (FSAP) was one element of that broader plan focusing on those products considered as "non-federally registered" sector and conservatively makes up 70 percent of the food Canadians consume.

The FSAP encompassed a series of initiatives to modernize and strengthen Canada's food safety system over a period of five years. To attain a better understanding of the food safety risks that Canadians may be exposed to, the CFIA was targeting and profiling commodities in the non-federally registered sector. In continuation of the FSAP, the CFIA needs to conduct surveys to determine the background levels of contamination in certain targeted food areas. The CFIA is looking for commercial laboratories to provide analytical testing services in food products. The results of the testing will be used by CFIA to determine the food safety risk to Canadians and identify areas where food safety issues may need to be addressed. The targeted Hazards are listed in Appendix I to Annex A, Reference Methods and Criteria. Each Hazard represents a specific Survey.

In addition, the CFIA may be required to take regulatory action under any or all of the Acts it administers or enforces by virtue of section 11 of the *Canadian Food Inspection Agency Act*, or under any other applicable law, on the basis of any information received or obtained in the course of performing the Work under this contract.

6.0 Scope

The Contractor must provide the following services:

6.1 Sample collection

The Contractor must collect and transport samples detailed in the Surveys for allergens, chemical additives and residue contaminants in food. The samples for these Surveys must be collected by the Contractor from the areas across Canada as identified in article 7.0 Tasks and Technical Specifications.

6.2 Analytical testing

The Contractor must perform analysis, for allergens, chemical additives and residue contaminants in food in accordance with the Survey established by the CFIA. A Survey similar to Appendix II to Annex A, Annual Survey Sample Plan Template will be provided by the Technical Authority following contract award. The Work must be performed in accordance with the "Date Planned" in the Annual Survey Sample Plan. For each following contracting year, the Technical Authority will provide to the Contractor a confirmation and details of the Survey to be conducted. Sample preparation and analysis must be performed at the location(s) detailed in the contract.

6.3 Procedures

The Contractor must provide the sample collected in accordance with a Survey Sample Plan. Refer to Appendix II, Annual Survey Sample Plan Template as an example. The Contractor must ship and handle samples in accordance with the procedures outlined in Appendix V, Sample Storage and Shipping Criteria.

The Contractor must perform analysis for allergens, chemical additives and residue contaminants in food on these samples collected in accordance with analytical methods and standard operating procedures (SOP). These SOPs must be accredited by the SCC in the Program Specialty Area for Agriculture and Food Products, http://www.scc.ca/sites/default/files/migrated_files/DLFE-453.pdf, or by the CALA, http://www.cala.ca, or were accepted by the Technical Authority. In the event SOPs are not accredited by SCC or CALA at the time of bidding, the accreditation must be achieved within a year of contract award, or the Contractor must cease all activities related to these SOPs immediately and advise the Technical Authority in writing until further instructions are received from the Technical Authority.

6.3.1 The Contractor must maintain current SOPs for any of the analytical areas of testing covered under the Contract. A copy of any revised SOP must be provided to the Technical Authority for approval within ten (10) working days, whenever a revision occurs. The analytical methodology to be used in the testing services provided by the Contractor must be as described in the Contractor's SOPs.

6.3.2 The Specific Reference Methods to be utilized as the basis for the performance of the Work are identified in Appendix I to Annex A, Reference Methods and Criteria. Any proposed method is a revision to a specific Reference Method and must be submitted to the Technical Authority for approval and must meet or exceed the criteria listed in Appendix I to Annex A, Reference Methods and Criteria.

6.3.3 The Contractor must demonstrate to the Technical Authority that the submitted Reference Method(s) is (are) validated in accordance with the Contractor's validation guidelines for all

sample matrices that are significantly different from those described in the Reference Methods. Validation records must be available for assessment by the Technical Authority upon request. The method performance parameters (LODs / LOQs) must be evaluated and must be stated where necessary according to the specific matrices if different.

6.3.4 The Contractor must not, in any event, allow the proposed revised Reference Method to be utilized until it is reviewed and approved by the Technical Authority. If the Contractor cannot perform the Work using the Reference Method originally identified, all work requiring that particular Reference Method must cease immediately. The Contractor must, as soon as possible, give notice to the Technical Authority of the reason for replacing the Reference Method and provide supporting documentation for this proposed Reference Method.

6.4 Turn around time for testing allergens, chemical additives and residue contaminants in food:

(a) Twenty (20) working days from receipt of the sample(s) for all Surveys except Undeclared Allergen Suveys

(b) Ten (10) working days from receipt of the sample(s) for Undeclared Allergen Surveys, including Multiple and Single Allergen Survey.

6.5 Sample retention

After all the required testing on a specific sample is completed and reported, the Contractor's laboratory must continue to hold any remaining sample material under frozen conditions to prevent spoilage, for an additional ninety (90) calendar days. This is necessary to allow for additional testing of the sample, when requested by the Technical Authority in accordance with 11.4.4 Additional Testing. CFIA will assume the costs for any additional testing. After ninety (90) calendar days, if no additional action has been requested by the Technical Authority, the remaining sample portions may be disposed of in accordance with the applicable federal, provincial and municipal laws and regulations.

7.0 Tasks and Technical Specifications

The Contractor must provide analytical services in accordance with the Survey established by the CFIA, as described in 8.0 Responsibilities of Canada. The Contractor is responsible for, but not limited to, performing the following tasks:

7.1 Sample collection and transportation

For each Survey listed in Appendix I to Annex A, Reference Methods and Criteria, CFIA projects a certain number of samples to be collected and tested over a 12 month period. These numbers are described as "Number of Samples estimated" in Table 1, Survey Sample Guidance. Samples will primarily be obtained at the retail level. These may include, but not be limited to samples from Grocery Stores, U picks, farmer's markets, ethnic stores, specialty stores, coffee/tea houses, juice bars. The Technical Authority will provide detailed Survey Sample Plan in a format similar to Appendix II to Annex A, Annual Survey Sample Plan Template to the Contractor after contract award as specified in 8.0 Responsibility of Canada. For each following contracting year, the Technical Authority will provide to the Contractor confirmation and details of the Survey to be conducted by the Contractor.

Table 1 Survey Sample Guidance

Survey Name	Yearly Number of samples estimated	Targeted product origin	Targeted product types including but not limited to:

Survey Name	Yearly Number of samples estimated	Targeted product origin	Targeted product types including but not limited to:
Acrylamide in selected foods	750	Domestic and imported	Breakfast cereals, cookies, crackers/croutons/crisp breads, Granola/cereal Bars, Infant biscuits, jarred infant food, Olives, pre-cooked/par-cooked sweet Potato products, Prune-based foods, seed and nut butters, soft breads, corn and corn products, vegetable chips/sticks.
Aflatoxins in selected foods	1000	Domestic and imported	Corn-based products, and nutmeats (peanuts, pistachios, brazil nuts, almonds, cashews, walnuts, hazelnuts, pecans, macadamia nuts), nut butters, dried fruits, spices and cocoa powder, breads (plain, containing grains, containing fruits) (may require method development/validation), breakfast/infant cereals (with grains, containing fruits, nuts) (many require method development/validation).
Arsenic Speciation in selected foods	1000	Domestic and imported	Apple Cider; bottled water, seaweed and seaweed products, breakfast/infant cereals (rice-based and no-rice-based), Rice and rice products, Fruit products (juices, blends, nectars, snacks, sauces), wheat bran, infant formula.
Coumarin in selected foods	750	Domestic and imported	Cinnamon, cinnamon containing spice mixes, cinnamon-containing food (i.e. cereals, granola bars, snack foods, and desserts, baking mixes, and baking goods).
Food Colours in selected foods	1000	Domestic and imported	Children's breakfast cereals, children's novelty snacks, Energy/sports drinks and Cocktail Mixes, Frozen Non-dairy novelties/frozen cheese cakes/pies/pastries, Pickled vegetables/Relish, Shelf-stable snacks/pastries, yogurt.
Fumonisins in selected foods	750	Domestic and imported	Corn and corn-based products (specifically meal, flour, grits, polenta, and extruded maize), Soy-based products, Selected cereal grain products, breakfast/infant cereals.
Furans (including 2-methyl and 3-methyl furan) in heat-treated foods	500	Domestic and imported	Breakfast cereals, snacks (i.e. pretzels, chips, crackers), canned fruits/vegetables, coffee, fruit juice, jarred infant foods, cooking sauces, canned soups/stews.
Glycoalkaloids in Potatoes	500	Domestic and imported	Potato tubers, potato products.
Multi-Mycotoxin Analysis in selected foods	750	Domestic and imported	Grain-based products; i.e. milled grains, bran/germ, meals, and flour of wheat, corn and oat, Finished grain-based products (e.g. breakfast/infant cereals, breads, cookies, crackers, baking mixes, noodles).
PBDEs in selected foods	500	Domestic and imported	Dairy products, vegetable oil/fat/nut butter; grains and milled grain products.
Perchlorate in selected foods	500	Domestic and imported	Dairy products (milk, cheese, yogurt, milk powder), infant formula (powdered formula, ready to use formula); fresh fruit and vegetables (cantaloupe, watermelon, apples, grapes, oranges, strawberries, spinach, salad greens, broccoli, collards, cucumber, green beans, celery), Grain products, Processed fruit and vegetables.

Survey Name	Yearly Number of samples estimated	Targeted product origin	Targeted product types including but not limited to:
PFOS/PFOA in selected foods	500	Domestic and imported	TV dinners, cereals, popcorn, flour, Fresh potatoes
Phthalates in selected foods	750	Domestic and imported	Bottled water, Ready-to-drink beverages, juice, drink, cereal products, Infant foods and formula, Prepackaged Meals, Jam, Bread and bakery products (may require method development/validation); dairy products (may require method development/validation), oil and fats (may require method development/validation)
Undeclared multiple Allergens in pre-packaged foods	1200	Domestic and imported	Flavour packets, spreads, pickled foods, snack foods, beer and wine, jarred infant foods, snacks (i.e. chips, pretzels, and crackers), processed fruits and vegetables, cooking sauces, Canned food, soup bases, broth, and grain-based products.
Undeclared single Allergen in pre-packaged foods	2000	Domestic and imported	Jarred infant foods, snacks (i.e. chips, crackers), Processed fruits and vegetables (i.e. canned fruit, juices/nectars), cooking sauces, canned food, infant cereals and foods, grain-based products (i.e. milled grains, bran/germ, meals, and flours of wheat, corn, oat and other grains), finished grain-based products, preserves, frozen foods, desserts/snacks and yogurts, ready to eat meals.

Sample numbers listed in Table 1 are provided only as an estimation for planning purposes and are not to be construed as final. Actual numbers may vary depending on the CFIA priorities at the time. These "estimated" samples may be requested from one to all years during the contract period.

7.1.2 Collection of samples will be required from six metropolitan areas: Calgary, Halifax, Montreal, Ottawa, Toronto, and Vancouver. No more than 10% of the samples collected will require travel between the city limits and the 100 KM radius. For any Survey listed in Table 1, proportions of samples collected from each of the 6 areas are estimated as follows:

Calgary: 12% of total number of a Survey
Halifax: 7% of total number of a Survey
Montreal: 23% of total number of a Survey
Ottawa: 7% of total number of a Survey
Toronto: 32% of total number of a Survey
Vancouver: 19% of total number of a Survey

7.1.3 Samples must be collected as prepackaged products unless stated otherwise. Where a bulk sample must be collected, the sample must be packaged individually to avoid direct contact from shipping and-or other material in the same shipping container and to ensure the integrity and traceability of the collected product.

7.14 A sample submission form in PDF format must accompany each sample collected for the Survey. The form template provided as Appendix III to Annex A, Sample Submission Form must be used.

7.1.5 Digital photos must be taken for each sample before it is unpacked. Photo submission requirements:

- A minimum of 2 digital photos in JPG format must be provided for each sample in accordance with Appendix IV, Requirements for Sample Photos.
- Photos along with Sample Submission Forms must be submitted electronically every two weeks, or mailed in CD/DVD to the Technical Authority.
- For Undeclared Multi-Allergen Survey, sample photos must be received and reviewed by the Technical Authority before it is unpacked or processed for analytical testing. Detailed requirement for these photos is described in Appendix IV to Annex A, Sample photos.
- In some cases, the Technical Authority may request additional sample photo(s) for clarification or investigation, or the Technical Authority may request photos before the date planned.
- The photos and the Sample Submission Form must be submitted to the Technical Authority before or at the same time as the testing results. The Technical Authority will reject result(s) submitted without photos or a sample submission form; charges associated with this sample will not be accepted.

7.1.6 If a sample cannot be collected in accordance with the Survey Sample Plan, the Contractor must contact the Technical Authority by e-mail to get further instructions. Results reported ten (10) working days behind the date planned will not be accepted by the Technical Authority, unless agreed upon in writing by the Technical Authority.

7.2 Where the Contractor proposes to subcontract sample collection services, the Contractor must submit details to the Technical Authority for review. Sample collection activity must not commence until the Technical Authority approves the subcontracting in writing.

7.3 Sample reception and analytical testing

7.3.1 Following receipt of the sample(s) at the testing laboratory, the Contractor must provide the following services:

- Inspect the sample(s) in accordance with the Survey Sample Plan, as an example refer to Appendix II to Annex A, Annual Survey Sample Plan Template, i.e. city, commodity and Product Type.
- Samples must meet the description in the Annual Survey Sample Plan.
- In addition, the sample(s) must arrive at the Contractor's accredited laboratory using the method described in Appendix V to Annex A, Shipping and Storage Criteria. For any deviations from the Annual Survey Sample Plan or the Shipping and Storage Criteria, the Contractor must report to the Technical Authority and obtain clarification from the Technical Authority within 48 hours after sample arrives.
- Sample(s) that fail to meet the Annual Survey Sample Plan or the Shipping and Storage Criteria must be claimed as unfit and rejected from further chemical or allergen analysis.
- At any point of time during the contract period, a documented record of shipping and storage condition of a specific sample must be available for investigation by the Technical Authority. Results reported on unfit samples will be rejected.

7.3.2 Analytical results must be reported to the Technical Authority using the template provided in article 11.0 Deliverables.

7.4 Confirmation Procedures

The Contractor must perform valid mass spectral confirmation procedures acceptable to the Technical Authority. Acceptable mass spectral confirmation criteria and approaches can be found in the Official Journal of the European Communities, "Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results" http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:221:0008:0036:EN:PDF

7.5 Reporting of Results

7.5.1 The Contractor must deliver, the Monthly Sample Collection Report (report#1) and Monthly Results Report (Report #2) electronically in Microsoft Excel format, to the Technical Authority, as specified in Article 11.0, Deliverables.

7.5.2 Numerical results must be reported to the significant figures indicated in the Contractor's SOP for all levels greater than the limit of detection. Unless otherwise specified, the units for the reported values are to be in parts per million: i.e. ppm, mg/kg, or mg/L. When the analyte is not confirmed by an unambiguous confirmatory technique, then the numerical value is designated as "unconfirmed." Whenever the analyte is absent (i.e. levels less than the detection limit for the method), the result is reported numerically as zero, "0".

7.5.3 Reports of Analyses (RoA) must be available when requested and a copy must be submitted to the Technical Authority within five (5) working days following the date requested in writing by the Technical Authority.

8.0 Responsibility of Canada

8.1 The Technical Authority will provide a detailed Annual Survey Sample Plan similar to Appendix II to Annex A, Annual Survey Sample Plan Template, within 2 weeks of contract award. When a Survey is to commence during a contractual year, the Technical Authority will provide the Annual Survey Sample Plan within four (4) weeks before the Survey is planned to start. The Annual Survey Sample Plan provides details to the Contractor regarding:

(a) sample specifications, including amongst others the commodity and product type of samples for the Survey, approximate sample size, origin, areas to be collected as stated in Appendix II to Annex A, Annual Survey Sample Plan Template;

(b) specific allergens, chemical additives and residue contaminants required to be analyzed for each sample;

(c) the time of year that such results are expected to be reported.

For each following contracting year, the Contractor will receive from the Technical Authority a confirmation and details of the survey under a new Annual Survey Sample Plan as per Appendix II, Annual Survey Sample Plan Template to be conducted by the Contractor. The Annual Survey Sample Plan must not be construed as firm, as it is subject to change. The level of services in any Survey is only an approximation of requirements.

9.0 Constraints

9.1 The Contractor should participate in proficiency check sample programs where those programs are available from organizations such as Food Analysis Performance Assessment Scheme (FAPAS), Association of Analytical Communities (AOAC). The Contractor should submit to the Technical Authority a copy of the final report received from the proficiency testing provider and, where it is not obvious, the identity of the laboratory in the report must be made known.

9.2 The Technical Authority may, at its discretion, randomly submit blind check samples as part of the Plan. These samples will be used as a proficiency indicator of the Contractor. In the event of an unsatisfactory test determination, the Technical Authority will notify the Contractor to initiate an investigation and report on the aberration at no cost to Canada.

9.3 Third party access to the findings, records or data of preliminary or final test result information on the CFIA testing will not be permitted. Results are only to be released to the Technical Authority.

10.0 Inspection of Facilities

Representatives of the CFIA or agents of Canada may conduct a facility site visit and evaluation to verify that the technical capabilities and human and material resources of the Contractor are carried out as required by the Contract. For example, turnaround times, reporting requirements, confirmatory testing decisions and procedures, and data management criteria may be verified.

To the extent that participating laboratories undertake testing, the Contractor will agree to submit to and participate fully in inspections and audits as they occur.

11.0 Deliverables

11.1 Survey

The Contractor must deliver the Surveys in accordance with the Table 2, Survey Guidance per Contract Year. The Technical Authority will provide the Contractor confirmation of Survey after contract award for contract Year 1 and the following contract years. The sample collection and testing services must be delivered in accordance with article 6, Scope and article 7, Tasks and Technical Specification.

Table 2: **Survey Guidance per Contract Year**

Survey Name	Contract Year 1	Contract Year 2	Contract Year 3	Option Period 1 (one year)	Option Period 2 (one year)
Acrylamide in selected foods	√	√	Not Required	Not Required	Not Required
Aflatoxins in selected foods	√	√	√	√	√
Arsenic Speciation in selected foods	√	√	√	√	√
Coumarin in selected foods	√	√	√	√	√
Food Colours in selected foods	√	√	√	√	√
Fumonisins in selected foods	√	√	√	√	√
Furans (including 2-methyl and 3-methyl furan) in heat-treated foods	√	√	√	√	√
Glycoalkaloids in Potatoes	√	√	√	Not Required	Not Required
Multi-Mycotoxin Analysis in selected foods	√	√	√	√	√
PBDEs in selected foods	√	√	√	√	√
Perchlorate in selected foods	√	Not Required	Not Required	Not Required	Not Required
PFOS/PFOA	√	√	√	√	√

Survey Name	Contract Year 1	Contract Year 2	Contract Year 3	Option Period 1 (one year)	Option Period 2 (one year)
in selected foods					
Phthalates in selected foods	√	√	√	√	√
Undeclared multiple Allergens in pre-packaged foods	√	√	√	√	√
Undeclared single Allergen in pre-packaged foods	√	√	√	√	√

11.2 Access to Sample Submission Form and Results

The Contractor must provide a secure web-based access of the results no later than three (3) months after contract award. This web-based access must allow the TA to access and view copies of the submission form that accompany the sample, the results reported by the laboratory, and the details of the sample. The website must be searchable using the sample number that is assigned as described in Report#1 at section 11.3.1. The website must be secured so that only the Technical Authority is able to access this information. The information must be posted within ten (10) working days after the test is completed.

11.3 Reporting

Two (2) monthly reports must be delivered to the Technical Authority by the Contractor as follows: (1) Monthly sample information records – Report #1 (Monthly Sample Collection Report), and (2) the final analytical data – Report #2 (Monthly Results Report) for samples submitted for analysis to the Technical Authority. These reports must be submitted no later than ten (10) working days after the DatePlanned prescribed in the Annual Survey Sample Plan for review and acceptance using the Reports as indicated below. The Reports must use the field names as indicated in **bold** below, with no exceptions. See article 7.5.

11.3.1 Monthly Sample Collection Report, **Report#1**: This report must contain the following information for all samples received for the month:

(i) **SAMPLE_NO** – The sample number identified on the Form. This will correlate with an equivalent sample number in the Survey to be provided.

(ii) **Region** – This is identified in the Survey and will reflect the location of the sample that was sampled on the Form.

(iii) **PickupCity** – The name of the city where the sample is purchased.

(iv) **Commodity** – this will be dairy, egg, meat, honey, fresh or processed depending on the sample.

(v) **DOM_IMP** – This will be either Domestic or Import depending on the source of the sample.

(vi) **Origin** – This is a three letter country code that matches the country of origin for the sample. A table of the country codes to use will be provided to the Contractor.

(vii) **Plan_Code** – This is provided in the Survey for each sample.

(viii) **ProductType** – This is provided in the Survey for each sample.

(ix) **Sample_Type** – This is a description or common name of the sample provided in the Form. In the case of any ambiguity, the Technical Authority will be consulted.

(x) **EST_NO** – This information to be filled in where the information is available on the Form.

(xi) **DateSample** – Date the sample was picked up, this will be on the Form.

(xii) **Perishable** – This will be either Y or N**.**

(xiii) **BrandName** – The brand name of the product.

(xiv) **SampleSize** – A numeric value of the sample size.

(xv) **SampleSizeUnit** – The unit used for the sample size. This can be g (gram), kg (kilogram) or other.

(xvi) **LotNo** – The lot number of the sample.

(xvii) **BestBefore** – The Best Before date described on the product package. This date should be entered in the format of DD/MM/YYYY**.**

(xviii) **PurchasedAt** – The name of the store where the sample is purchased.

(xix) **PurchasedAtAddress** – The address of the store where the sample is purchased**.**

(xx) **ContainerType** – The type of the container used for sample package.

(xxi) **UPC** – The barcode printed on the sample label.

(xxii) **Store_Type** – The type of the store where a sample is purchased. This should be correlated to Survey specification.

(xxiii) **DateRecd** – The date the sample is received by the Contractor's laboratory in the format of DD/MM/YYYY**.**

(xxiv) **Lab_Code** – This code will be assigned to the Contractor's laboratory by CFIA to be used on all reports.

(xxv) **Comment** – Report any deviations of the sample from the Survey, such as change of country of origin, region is different, guidance provided by the Technical Authority.

(xxvi) **Photos** – If submitted to the Technical Authority, enter “Y”, if not, enter “N”.

(xxvii) **Form** – If submitted to the Technical Authority, enter “Y”, if not, enter “N”.

11.3.2 Monthly Results Report, **Report #2 :** This report must contain the following information for all results reported for the month:

(i) **SAMPLE_NO** – See Report #1 above.

(ii) **Commodity** – See Report #1 above**.**

(iii) **Tissue** – Animal tissue name for meat products (For example:. muscle, liver)., N/A for other products.

(iv) **Program** – The name of the CFIA Program which the test falls under and is identified in the Survey.

(v) **Analyte** – The name of the analyte being tested.

(vi) **Amount** – The amount of the analyte determined in ppm, unless otherwise specified.

(vii) **Date_Analyzed** – The date the analysis is conducted in the Contractor's laboratory.

(viii) **Date_Rept** – The date the result is reported.

(ix) **Invoice_No** – Invoice Number.

11.3.3 Ad hoc Report

The Contractor must submit Reports of Analysis (RoA), when requested in writing by the Technical Authority.

11.4 Task Authorization Work

The Contractor may be required to perform various tasks within the scope of the contract, on an "as and when requested" basis. An obligation of any work will come into force only when a Task Authorization is approved and issued in accordance with the clause entitled "Task Authorization Process".

11.4.1 Optional Survey Packages

The Contractor must perform additional sample collection and testing service for a Survey on an "as and when requested" basis in accordance with the option packages defined in Table 3, Optional Survey Packages during the contract period and the optional periods up to a maximum number of samples.

The Technical Authority will provide the additional Surveys to the Contractor no less than eight (8) weeks prior to sample collection by the Contractor. Sample collection will be carried out in similar way as Appendix II to Annex A, Annual Survey Sample Plan Template. The Reference Method and the criteria will be provided in accordance with Appendix 1 to Annex A. The scope will be in accordance with 6.1, 6.2, 6.3, 6.5, 11.2 and 11.3. The turnaround time for testing allergens, chemical additives and residue contaminants in food will be specified in the Task Authorization.

Table 3: **Optional Survey Packages**

Survey Name	**Option Package Number**	**Estimated Additional Number of Sample(s) above the number of samples in Table 1**
Aflatoxins in selected foods	1	1 - 1000

Survey Name	Option Package Number	Estimated Additional Number of Sample(s) above the number of samples in Table 1
Arsenic Speciation in selected foods	2	1 - 1000
Coumarin in selected foods	3	1 - 750
Food Colours in selected foods	4	1 - 1000
Fumonisins in selected foods	5	1 - 750
Furans (including 2-methyl and 3-methyl furan) in heat-treated foods	6	1 - 750
Multi-Mycotoxin Analysis in selected foods	7	1 - 750
PBDEs in selected foods	8	1 - 500
PFOS/PFOA in selected foods	9	1 - 500
Phthalates in selected foods	10	1 - 750
Undeclared multiple Allergens in pre-packaged foods	11	1 - 600
Undeclared single Allergen in pre-packaged foods	12	1 - 1000

11.4.2 Expert Testimony Services

The Contractor must provide Expert Testimony on an "as and when requested" basis. The CFIA may be required to take regulatory action under any or all of the Acts it administers or enforces by virtue of section 11 of the *Canadian Food Inspection Agency Act*, or under any other applicable law, on the basis of any information which the CFIA or its employees, officials, agents, or contractors may receive or obtain in the course of performing the Work or by any other means. Such regulatory action may be taken by or on behalf of the CFIA without any repercussion whatsoever from the Contractor to the CFIA. The Contractor may be called upon to act as Expert Witness at legal proceedings. Testimony or evidence may be required in relation to a food sample with the Contractor, including receipt, storage and disposal, and details of the standard operating procedures utilized to determine the possible test results, as well as all records of procedures. Travel may be required and must have prior authorization of the Technical Authority.

11.4.3 Additional Surveys Services

The Contractor must provide additional sample collection and testing services, on an "as and when requested" basis. The CFIA may be required to conduct additional Surveys to determine the background levels of additional allergens, chemical residues and residue contaminants in certain food areas. The results of the testing will be used by CFIA to determine the food safety risk to Canadians and identify areas where food safety issues may need to be addressed. The Technical Authority will provide the additional targeted Hazards and additional Surveys to the Contractor no less than eight (8) weeks prior to sample collection by the Contractor. Sample collection will be carried out similarly to Appendix II to Annex A, Annual Survey Sample Plan Template. The Reference Method and the criteria will be provided in a similar way to Appendix 1 to Annex A. The scope will be in accordance with 6.1, 6.2, 6.3 and 6.5, 11.2 and 11.3. The

turnaround time for testing allergens, chemical additives and residue contaminants in food will be specified in the Task Authorization.

11.4.4 Additional Analytical Testing Services

The Contractor must provide additional analytical testing services, on an “as and when requested” basis. The CFIA may be required to conduct additional testing on samples retained in accordance with article 6.5. The Reference Method and the criteria will be provided in a similar way to Appendix 1 to Annex A. The scope will be in accordance with 6.1, 6.2, 6.3 and 6.5, 11.2 and 11.3. The turnaround time for testing allergens, chemical additives and residue contaminants in food will be specified in the Task Authorization.

12. LANGUAGE REQUIREMENTS

All written and verbal communication between the Contractor and the Technical Authority must be in English

Appendix I to Annex A

Reference Methods and Criteria

The technical requirement is for:

1. An Analytical Method

An analytical method including the detailed SOP must be based upon the principles found in the Reference indicated in Table 4 Reference Methods and Criteria below. The columns of LOD / LOQ, and the Report of Result in Table 4 are mandatory requirements. It is not mandatory to utilize a particular manufacturer if specified in the Reference Method, however the equipment or supplies contained in the accepted SOP submitted by the Contractor must have the same or better technical specifications. The Detection Method as detailed in the Reference is not mandatory; however the Contractor must submit a method of detection in its SOP that meets the LOD/LOQ required for the Food Samples identified in Table 4.

2. A Confirmation Technique

With the exception of Undeclared Allergens and Aflatoxins, confirmation using an LC/MS technique, which is to be developed by the Contractor, is required for all samples found positive by the initial method. Aflatoxins require confirmation using an LC/MS technique for samples with levels in excess of 8 ppb by the initial method. Undeclared Allergens do not require a confirmation method, unless specified otherwise by the Technical Authority in writing. The confirmation technique must be included in the submitted SOPs for each Hazard. Laboratories may choose to opt for an initial detection method employing LC/MS, thus avoiding the non-specific LC/non-selective detection. If so, additional will not be required if the LC/MS technique provides for a minimum of 4 identification points as described in the Official Journal of the European Communities, "Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results" http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:221:0008:0036:EN:PD

Table 4 Reference Methods and Criteria

Hazard	Targeted Analyte(s)	Reference	Detection Method	LOD / LOQ Criteria*	Report Of Result**	Est. Rate of positives***
Acrylamide	Acrylamide	The Determination of Acrylamide in Foods by LC-ESI-MS-MS (see http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/res-rech/lps_003-eng.pdf or http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/res-rech/lps_003-fra.pdf	LC-ESI-MS/MS	LOD ≤ 5 ppb LOQ ≤ 15 ppb	The "ANALYTE" is to be reported as "Acrylamide" and the numerical value as the "AMOUNT", in ppb.	90%
Aflatoxins	Aflatoxin B1 Aflatoxin B2 Aflatoxin G1 Aflatoxin G2	Aflatoxins in Food Products - Immunoaffinity Column Method (Based on AOAC 990.33 and AOAC 991.31(2000)) *See Attachment 1 to Appendix 1 - Aflatoxins in Food Products*	HPLC with fluorescence detection	LOD ≤ 1 ppb LOQ ≤ 3 ppb	The "ANALYTE" is to be reported as "Aflatoxin Screen" and the "AMOUNT" is to be "0" for a negative and a "1" for a positive for one or more of the analytes. In the event of a positive, the analyte(s) found to be positive is/are to be reported as a separate entry and the amount as the actual value confirmed, in ppb.	10%
Arsenic Speciation	$As^{+3,}$ DMA, MMA, and As^{+5} in seaweed. AsC, AsB, $As^{+3,}$ DMA,	Speciation of Arsenic in a variety of Foods *See Attachment 2 to Appendix 1 - Speciation of Arsenic in a Variety*	LC-ICP-MS	See LODs and LOQs in Attachment 2 based on product types	The "ANALYTE" is to be reported as "As Speciation Screen" and the "AMOUNT" is to be "0" for a negative and a "1" for a positive for one or more of the analytes. In the event	10%

Hazard	Targeted Analyte(s)	Reference	Detection Method	LOD / LOQ Criteria*	Report Of Result**	Est. Rate of positives***
	MMA, and As^{+5} in other products	*of Foods*			of a positive, the analyte(s) found to be positive is/are to be reported as a separate entry and the amount as the actual value confirmed, in ng/ml.	
Coumarin	Coumarin (2H-Chromen-2-one)	Courmarin in Cinnamon Containing Food in HPLC *See Attachment 3 to Appendix 1 - Coumarin*	HPLC with Photodiode array detection	LOD ≤ 0.5 ppm LOQ ≤ 1.5 ppm	The "Analyte" is to be reported as "COUMARIN", the "AMOUNT" is to be a numerical value, in ppm.	95%
Food Colours	16 Water-Soluble food colours listed in Table 4 of Attachment 4; and 16 Fat-Soluble dyes listed in Table 1 of Attachment 5	Determination of Water-Soluble Colours by HPCL-RV-Visible (DAD) in Foodstuff *See Attachment 4 to Appendix 1 - Water Soluble Food Colours* Determination of Fat-Soluble Dyes in Foodstuff by HPLC *See Attachment 5 for Fat-Soluble dyes*	HPLC with Photo-Diode Array detector	Amaranth, Indigo Carmine, Sunset Yellow FCF, Fast Green FCF, Brilliant Blue FCF — LOD ≤ 0.10 ppm Allura Red, Ponceau SX, Erythrosin B, Ponceau 4R, Azorubine, Lissamine Green, Patent Blue Violet, Rhodamine B — LOD ≤ 0.20 ppm Tartrazine, Chlorophyllin, Quinoline Yellow — LOD ≤ 0.50 ppm	The "ANALYTE" is to be reported as "Colour Water-Soluble Screen" or "Colour Fat-Soluble Screen", the "AMOUNT" is to be "0" for a negative	95%

Hazard	Targeted Analyte(s)	Reference	Detection Method	LOD / LOQ Criteria*	Report Of Result**			Est. Rate of positives***
		in Food		Fat-Soluble dyes		LOD ≤ 0.03 ppm	and a "1" for a positive for one or more of the analytes. In the event of a positive, the food colour(s) found is/are to be reported in separate entry (or entries) and the amount as the actual value confirmed, in ppm.	
				LOQ ≤ 5 times of the LOD				
Fumonisins	Fumonisin B1 Fumonisin B2	*JOURNAL OF AOAC INTERNATIONAL VOL. 91, NO. 3, 2008. pp 598-606*	LC/MS	LOD ≤ 20 ppb LOQ ≤ 40 ppb	The "ANALYTE" is to be reported as "Fumonisin Screen" and the "AMOUNT" is to be "0" for a negative and a "1" for a positive for one or more of the analytes. In the event of a positive, the analyte(s) found to be positive is/are to be reported in separate entry			30%

Hazard	Targeted Analyte(s)	Reference	Detection Method	LOD / LOQ Criteria*	Report Of Result**	Est. Rate of positives***
					(entries) as Fumonisin B1 and/or Fumonisin B2 and the amount as the actual value confirmed, in ppb.	
Furans	2-methyl Furan 3-methyl Furan Furan	Development of an analytical method and survey of foods for furan, 2-methylfuran and 3-methylfuran with estimated exposure *Food Additives & Contaminants: Part A, 27:6, 764-775*	GC-MS	LOD ≤ 0.5 ppb LOQ ≤ 1.0 ppb	The "ANALYTE" is to be reported as "Furans Screen" and the "AMOUNT" is to be "0" for a negative and a "1" for a positive on one or more of the analyte (s). In the event of a positive, the analyte (s) found to be positive is /are to be reported as a separate entry as Furan and/or 2-methyle Furan and /or 3-Methyl Furan. The "AMOUNT" is as the actual value confirmed, in ppb	80%
Glycoalkaloids	Solanine Chaconine	Determination of "α-Solanine and α-Chaconine" in Potato tubers *See Attachment 6 to Appendix 1 - Glycoalkaloids Analytical Method*	Reversed-phase liquid chromatography with ultra violet detection.	LOD ≤ 0.2 mg/100g product weight LOQ ≤ 0.6 mg/100g product weight	The "ANALYTE" is to be reported as "Glycoalkaloids Screen" and the "AMOUNT" is to be "0" for a negative and "1" for a positive for Solanine and/or Chaconine. In the event of a positive, the analyte(s) found to be positive is/are to be reported as a separate entry (Solanine and/or Chaconine) and the "AMOUNT" as the actual value confirmed in mg/100g product weight,	60%

Hazard	Targeted Analyte(s)	Reference	Detection Method	LOD / LOQ Criteria*	Report Of Result**	Est. Rate of positives***
					and an additional entry for "Total Glycoalkaloids" as the sum of all Glycoalkaloids found, in mg/ product weight.	
Multi-Mycotoxin analysis	See Attachment 7	Multimycotoxin Analysis in Cereal Grains by HPLC-MS/MS *See Attachment 7 to Appendix 1 - Multimycotoxin Analysis*	HPLC-MS/MS	See details in Attachment 7	The "ANALYTE" is to be reported as "Multimycotoxin Screen" and the "AMOUNT" is to be "0" for a negative or below LOD and a "1" for a positive on one or more of the Mycotoxin(s). The individual toxin(s) found positive is/are to be reported as a separate entry/entries (i.e. Aflatoxin B1) and the "AMOUNT" as the actual value confirmed, in ug/g	10%
PBDEs	BDE-17, BDE-28, BDE-47, BDE-66, BDE-77, BDE-85, BDE-99, BDE-100, BDE-138, BDE-153, BDE-154, BDE-183, BDE-209	Polybrominated Diphenyl Ether (PBDE) Levels in an Expanded Market Basket Survey of U.S. Food and Estimated PBDE Dietary Intake by Age and Sex Environ Health Perspect. *2006 October; 114(10): 1515–1520* or Polybrominated Diphenyl Ethers	HR-MS/MS	LOD ≤ 40 pg/g LOQ ≤ 120 pg/g	The "ANALYTE" is to be reported as individual PBDE and its numerical value as the "AMOUNT", in pg/g	95%

Hazard	Targeted Analyte(s)	Reference	Detection Method	LOD / LOQ Criteria*	Report Of Result**	Est. Rate of positives***
		(PBDEs) in Foodstuffs:Human Exposure through the Diet *J. Agric. Food Chem.* 2003, *51,* 3191-3195 3191				
Perchlorate	Perchlorate	Estimated Dietary Exposure of Canadians to Perchlorate through the Consumption of Fruits and Vegetables Available in Ottawa Markets *J.Agric.Good Chem. 2009, 57, 9250-9255*	IC-MS/MS	LOD ≤ 1.0 ppb LOQ ≤ 3.0 ppb	The "ANALYTE" is to be reported as "Perchlorate" and the numerical value as the "AMOUNT", in ppb	50%
PFOS/PFOA	PFOS PFOA	Dietary Exposure of Canadians to Perfluorinated Carboxylates and Perfluorooctane Sulfonate via Consumption of Meat, Fish, Fast Foods, and Food Items Prepared in Their Packaging *J. Agric. Food Chem. 2007, 55, 32̃033210*	LC-MS/MS	LOD ≤ 1 ppb LOQ ≤ 3 ppb	The "ANALYTE" is to be reported as "FR Screen" and the "AMOUNT" is to be "0" for a negative or below LOD and a "1" for a positive on PFOS and /or PFOA. The individual compound(s) found positive is/are to be reported as a separate entry/entries The "ANALYTE" is to be reported as "PFOS" and / or "PFOA" (in separate entries) and the numerical value as the "AMOUNT", in ppb	5%

Hazard	Targeted Analyte(s)	Reference	Detection Method	LOD / LOQ Criteria*	Report Of Result**	Est. Rate of positives***
Phthalates	BBP, DBP, DEHP, DNOP, DINP, DIDP	A Determinative and Confirmatory method for Phthalate Esters in Foods by LC-MS/MS *See Attachment 8 to Appendix 1 - Phthalate Esters in Food*	LC-MS/MS	LOD ≤ 0.5 ppm LOQ ≤ 1.0 ppm	The "ANALYTE" is to be reported as "Phthalates Screen" and the "AMOUNT" is to be "0" for a negative or below LOD and a "1" for a positive on one or more of the Phthalate(s). The individual compound found positive is/are to be reported as separate entry/entries (i.e. BBP) and the "AMOUNT" as the actual value confirmed, in ppm.	1%
Undeclared Multiple Allergens	See Appendix I (A)	*See Appendix I (A)*	*See Appendix I (A)*	*See Appendix I (A)*	The "ANALYTE" is to be reported as one of the following: Allergen-Peanut. Allergen-Almond, Allergen-Egg, Allergen-Casein, Allergen-BLG, Allergen-Hazelnut, Allergen-Soy Allergen-Sesame, Allergen-Gluten, Allergen-Mustard The amount is to be reported as "0" for a negative and the quantitative amount in ppm for positives.	20%
Undeclared Single Allergen	See Appendix I (A)	*See Appendix I (A)*	*See Appendix I (A)*	*See Appendix I (A)*	The "ANALYTE" is to be reported as the single Allergen requested. The amount is to be	5%

Hazard	Targeted Analyte(s)	Reference	Detection Method	LOD / LOQ Criteria*	Report Of Result**	Est. Rate of positives***
					reported as “0” for a negative and the quantitative amount in ppm for positives.	

* LOQ and LOD are for individual analyte when more than one is targeted.
** For Surveys with single targeted analyte, results below LOD are to be reported as a numerical value 0.
*** These rates are estimates only, given in good faith, and are not to be taken as an indication of the actual rate of positives that will be found in the samples received by the laboratories.

APPENDIX I (A)
ALLERGEN TESTING KIT SPECIFICATIONS

The testing will be for Undeclared Allergens – Multiple or Single. For Undeclared Multi-Allergens Survey, analysis will be carried out only on allergens not declared on the ingredient list. (For Multi-Allergens Survey, an average of seven (7) or eight (8) allergens are estimated per sample). CFIA will assign the allergens to be tested after reviewing sample information, i.e. the Form and sample photos submitted by the contractor. For Undeclared Single Allergen, test will be carried out only on the allergen prescribed in the survey. All testing must be performed with commercial enzyme-linked immunosorbent assay (ELISA) based methods that quantitate an allergenic protein or allergenic protein marker that meet the following specifications:

Food Allergen	**Criteria**
Peanut	Must use a commercial ELISA test kit that has been validated under the Performance Tested Methods Program of AOAC. www.aoac.org/testkits/testedmethods.html
Almond	Must be a quantitative assay with manufacturer calibration to 2.5 ppm or less (LOQ).
Egg	Must be a quantitative assay with manufacturer calibration to 2.5 ppm or less (LOQ). The antibodies must have applicability to the analysis of unprocessed and heat processed egg in food products.
	Samples with milk content tested positive by Neogen Veratox® for Egg Allergen needs confirmation by another testing kit.
Milk	Must quantitate both casein and ßeta-lactoglobulin individually.
i) Casein	The assay must be a quantitative assay and have a manufacturer calibration to 1.0 ppm or less (LOQ).
	When report, do not convert Casein content to total Milk content.
ii) Beta-Lactoglobulin	The assay must be a quantitative assay and have a manufacturer calibration to 0.1 ppm or less (LOQ).
Hazelnut	Must be a quantitative assay with manufacturer calibration to 2.5 ppm or less (LOQ).
Soy	The assay must be capable to detect soy residues, including isolates, concentrates, flours, textured soy and soy flakes at various stages of processing.
	The assay must be a quantitative assay and have a manufacturer calibration to 2.5 ppm (soy flour scale) or less (LOQ).
Sesame	The assay must be a quantitative assay and have a manufacturer calibration to 0.5 ppm or less (LOQ) of Sesame Seed Protein
Gluten	Must use a commercial ELISA test kit that has been validated under the Performance Tested Methods Program of AOAC. www.aoac.org/testkits/testedmethods.html
	The assay must meet the requirements of Codex Alimentarius Standard 118, CODEX Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten, section 5.2 Method for determination of gluten www.codexalimentarius.net/web/standard_list.jsp
Mustard	The Assay must be a quantitative assay and have a manufacturer calibration to 2.5 ppm or less of LOQ of mustard protein.

APPENDIX II TO ANNEX A
ANNUAL SURVEY SAMPLE PLAN TEMPLATE

Aflatoxins in Corn Products, Nut Products, Raisins, Spices, and Cocoa

SAMPLE NUMBER	City	PickupProv	DATEPlanned	PLAN_CODE	DOM_IMP	COMMO-DITY	PRODUCT-TYPE	Description	STORE_TYPE	ORIGIN	LAB_CODE	program	analyte	Tissue
C2014AFLA00001	CALGARY	AB	01-Mar-14	2014_SB433	IMPORT	IMFD	Corn Products	Corn Tacos	Specialty/Bulk	CHN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00002	CALGARY	AB	01-Mar-14	2014_SB433	IMPORT	IMFD	Corn Products	Corn Tacos	National Chain	CHN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00003	CALGARY	AB	01-May-14	2014_SB433	IMPORT	IMFD	Popcorn	Popcorn	National Chain	TWN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00004	MONTREAL	QC	01-May-14	2014_SB433	IMPORT	IMFD	Chips - Tortilla/Corn	Chips - Tortilla/Corn	National Chain	TWN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00005	MONTREAL	QC	01-Jun-14	2014_SB433	IMPORT	IMFD	Nutmeats	Peanut	Specialty/Bulk	GBR	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00008	MONTREAL	QC	01-Jun-14	2014_SB433	IMPORT	IMFD	Butter - Nut	Other Nut Butters	National Chain	USA	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00009	MONTREAL	QC	01-Jul-14	2014_SB433	IMPORT	IMFD	Popcorn	Popcorn	Specialty/Bulk	USA	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00010	MONTREAL	QC	01-Jul-14	2014_SB433	IMPORT	IMFD	Popcorn	Popcorn	National Chain	USA	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00011	OTTAWA	ON	01-Jul-14	2014_SB433	IMPORT	IMFD	Popcorn	Popcorn	National Chain	USA	CFIA	AFLATOXINS	Aflatoxin Screen	N/A

C2014AFLA00012	OTTAWA	ON	01-Jul-14	2014_SB433	IMPORT	IMFD	Chips - Tortilla/Corn	Chips - Tortilla/Corn	Local/Regional	USA	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00013	OTTAWA	ON	01-Jul-14	2014_SB433	IMPORT	IMFD	Chips - Tortilla/Corn	Chips - Tortilla/Corn	National Chain	USA	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00014	OTTAWA	ON	01-Jul-14	2014_SB433	IMPORT	IMFD	Nutmeats	Pistachio	National Chain	USA	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00015	OTTAWA	ON	01-Aug-14	2014_SB433	IMPORT	IMFD	Raisin	Raisin	Specialty /Bulk	USA	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00016	VANCOUVER	BC	01-Aug-14	2014_SB433	IMPORT	IMFD	Cocoa Powder	Natural Cocoa	Local/Regional	USA	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00017	VANCOUVER	BC	01-Aug-14	2014_SB433	IMPORT	IMFD	Cocoa Powder	Natural Cocoa	Specialty /Bulk	USA	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00021	VANCOUVER	BC	01-Sep-14	2014_SB433	DOMESTIC	PROCESSED	Cocoa Powder	Dutch Process Cocoa	National Chain	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00022	VANCOUVER	BC	01-Sep-14	2014_SB433	DOMESTIC	PROCESSED	Butter - Nut	Almond Butter	Local/Regional	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00023	VANCOUVER	BC	01-Sep-14	2014_SB433	DOMESTIC	PROCESSED	Butter - Nut	Hazelnut Butter	Local/Regional	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00024	VANCOUVER	BC	01-Sep-14	2014_SB433	DOMESTIC	PROCESSED	Butter - Nut	Peanut Butter	Local/Regional	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00025	VANCOUVER	BC	01-Sep-14	2014_SB433	DOMESTIC	PROCESSED	Butter - Nut	Peanut Butter	Local/Regional	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00026	TORONTO	ON	01-Sep-14	2014_SB433	DOMESTIC	PROCESSED	Butter - Nut	Other Nut Butters	Specialty /Bulk	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A

C2014AFLA00029	TORONTO	ON	01-Nov-14	2014_SB433	DOMESTIC	PROCESSED	Raisin	Raisin	Local/Regional	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00030	TORONTO	ON	01-Nov-14	2014_SB433	DOMESTIC	PROCESSED	Raisin	Raisin	Specialty/Bulk	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00031	TORONTO	ON	01-Nov-14	2014_SB433	DOMESTIC	PROCESSED	Spices	Chili Powder	Specialty/Bulk	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00032	TORONTO	ON	01-Nov-14	2014_SB433	DOMESTIC	PROCESSED	Spices	Paprika	Local/Regional	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00033	TORONTO	ON	01-Nov-14	2014_SB433	DOMESTIC	PROCESSED	Corn Products	Corn Tacos	National Chain	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00034	HALIFAX	NB	01-Dec-14	2014_SB433	DOMESTIC	PROCESSED	Corn Products	Corn Tacos	Specialty/Bulk	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00035	HALIFAX	NB	01-Dec-14	2014_SB433	DOMESTIC	PROCESSED	Popcorn	Popcorn	National Chain	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00036	HALIFAX	NB	01-Dec-14	2014_SB433	DOMESTIC	PROCESSED	Popcorn	Popcorn	Specialty/Bulk	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00037	HALIFAX	ON	01-Dec-14	2014_SB433	DOMESTIC	PROCESSED	Butter - Nut	Other Nut Butters	Specialty/Bulk	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00038	HALIFAX	ON	01-Dec-14	2014_SB433	DOMESTIC	PROCESSED	Raisin	Raisin	National Chain	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A

C2014AFLA00042	HALIFAX	ON	01-Dec-14	2014_SB433	DOMESTIC	PROCESSED	Raisin	Raisin	Specialty/Bulk	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00043	HALIFAX	ON	01-Dec-14	2014_SB433	DOMESTIC	PROCESSED	Raisin	Raisin	Local/Regional	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A
C2014AFLA00070	HALIFAX	ON	01-Dec-14	2014_SB433	DOMESTIC	PROCESSED	Spices	Chili Powder	Local/Regional	CAN	CFIA	AFLATOXINS	Aflatoxin Screen	N/A

APPENDIX II(A) TO ANNEX A
TEMPLATE FOR SURVEY INFORMATION SPECIFIC FOR SAMPLERS

Refer to the detailed sample plan Excel spreadsheet for comprehensive sampling requirements including:

- Sample number
- Product type
- Country of origin
- Sampling location (store type and city)

These sampling requirements are vital to the validity of the Survey. **No substitutions or alterations** of product type, sample number, country of origin, or location will be allowed. If unable to locate the product specified, please contact: FSAPchemistryTS@inspection.gc.ca

Unless stated otherwise for a specific commodity type, sample:

- Largest number of brands possible
- Fair trade, premium, generic, organic and non-organic products
- Domestic and imported products
- As many different countries of origin/manufacture as possible
- All available packaging types (For example: pre-packaged, plastic, glass)

Refer to the **General Sampling Instructions – Food Safety Action Plan (FSAP) Chemistry Targeted Surveys** document for more information regarding the selection, sampling, shipping, and recording of sample details for each product.

Sampling Instructions:

- Do not sample open, broken or damaged products.
- Do not sample products that are past the “use by” date or the “best before” date.
- Collect samples so that they can be tested before the “use by” or “best before” date.
- Please send samples in their original packaging to the laboratory.
- Please clearly photograph each product with sample identification number attached. The picture(s) must plainly show:
    - Manufacturer/company name
    - Brand Name
    - Product Type
    - Ingredients
    - Sample Number
    - Country of Origin
- The picture filename must be identical to the sample number. Details of sample photos are specified as Appendix IV to Annex A, Requirement of Sample Photos.
- A Sample Submission Form must be filled out for and accompany each sample (see 7.1.4). It is imperative that the country of origin/processing/packaging and/or the importer address be clearly identified on the sample form. Describe the sample brand/type/flavour in as much detail as possible. Include the lot number (stamped in ink on box, carton or can) and/or expiry date of the product if available.
- Please store sample submission forms and pictures electronically.
- Ship samples so that they arrive intact.
- Ship refrigerated items with ice packs and frozen samples with freezer packs.
- **Do not sample from bulk bins.**

Survey Title Aflatoxins in Corn Products, Nut Products, Raisins, Spices, and Cocoa
Sample Plan Code: 2014_SB433
Sample ID Numbers: C2014AFLA00001-C2014AFLA01000

Sample a minimum of 1000 g of product OR 5 packages of product

Commodity	DO Sample	DO NOT sample
Corn products	o Corn cereals o Corn tacos o Tortilla/corn chips o Popcorn (popped and unpopped)	o Mixed cereals containing corn o Wheat flour based tortillas o Caramel or other flavoured popcorn
Nutmeats	o In-shell, shelled, roasted, seasoned nuts o Almonds o Brazil nuts o Peanuts o Pistachios o Walnuts	o Mixed nuts o Candied nuts o Trail mix containing nuts o Party mixes
Nut butters	o Almond o Hazelnut o Peanut o Other nut butters (e.g. pistachio, macadamia)	o Chocolate or flavoured nut butters o Soy nut butter o Other nut alternatives
Raisins	o Golden o Sultanas o Thompson o Currants	o Mixed products containing raisins (trail mixes etc.)
Spices	o Chili powder o Paprika (Hot, Hungarian, Plain, Smoked, Spanish, Sweet)	o Spice mixes
Cocoa Powder	o Natural o Dutch Process	o Hot chocolate mixes

Appendix III to Annex A
Sample Submission Form

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

FOOD SAFETY ACTION PLAN (FSAP) SAMPLE GATHERING INFORMATION

2013-2014 Targeted Surveys. SAMPLE PLAN: 2013_AB123

IMPORT CHEM	IMPORT MICRO	DOMESTIC CHEM	DOMESTIC MICRO

Date Sampled: July 01, 2013

Sample # (please enter the full ID): C2013ABCD01234

Pick Up Lab: Canadian Food Inspection Agency (CFIA)

Contractor Lab: XXXXX LABORATORY SERVICES LTD.

Retail Location: (Complete Name and Address)
XXXXX GROCERY STORE
1234 MAIN STREET, OTTAWA, ON A1B 2C3

Product Description & Universal Product Code (UPC) (if applicable): ORANGE JUICE

Product Name: ANY CANNED JUICE

Brand Name: XXXXX

Country of Origin: CANADA

Shipment Tracing Number
1234567890

LOT #: 012345678

Best Before Date: 31 JL 2013

Unit size: 2 X 250 ML

Container type: PLASTIC BOTTLE

Grower / Imported By / Packed By / Distributed By address:
xxxxxxxxxxxxxxxxxxxxxxxxx

	Phone#:
Sampled By: JOHN DOE	**613-XXX-XXXX**
Lab Sample Tracking System (LSTS) system #	
Date Received:	**Temperature on receipt:**

Please print clearly

Appendix IV to Annex A
Requirement for Sample Photos

At least 2 digital photos of every sample must be taken and forwarded to the Technical Authority before the sample is to proceed for analysis. Additional sample photo(s) will be requested when details of the sample are not captured. Photo size is between 1600 X 1200 ppi and maximum 2592 X 1944 ppi.

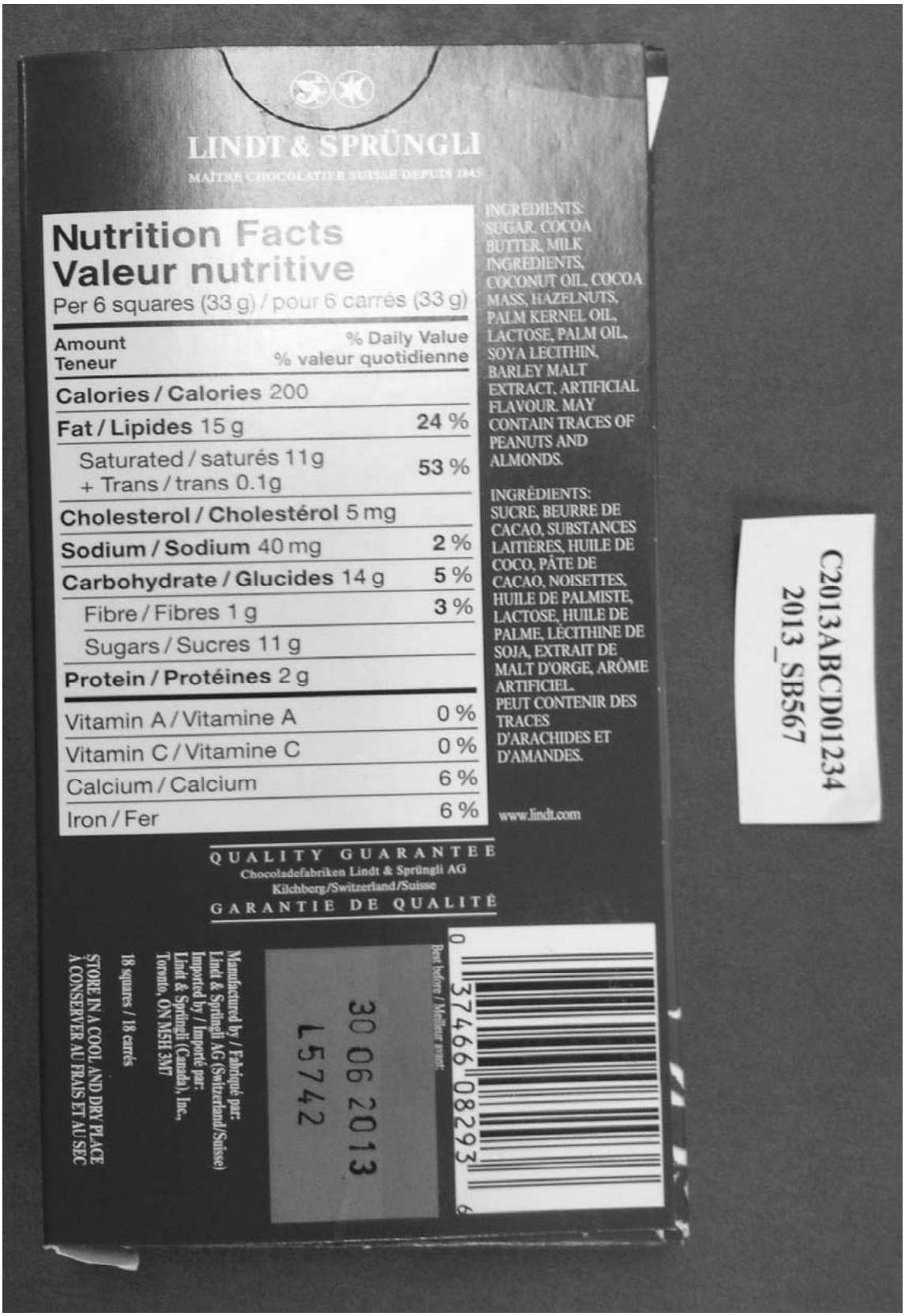
- 1 or more photo must be able to show the entire sample, including the packaging;
- 1 or more photos must be able to show sample number and Plan code (marked or labeled by the sampler) along with the sample package.
- 1 or more photos must be able to show clearly the product information printed on the product, i.e. Brand, lot number, expiry date, ingredient, and etc.
- All photos must be in jpg format. Photo files must be named with Sample Number in the beginning, followed by letter(s) at the end to identify the side of the package. In case of more than 1 photo are taken from one side, add number at the end. i.e. C2014ABCD12345_F1.jpg
  - 'F' for Front view
  - 'B' for Back view
  - 'L' for Left view
  - 'R' for Right View
  - 'T' for Top view
  - 'BM' for Bottom view
- Boxed items may need up to seven pictures to capture all sides (Front, Back, Left, Right, Top, Bottom and entire box)
- Submitted sample photos are somewhat expected to be similar to the ones below. The quality of the photos must be good enough to get any required information including UPC and LOI if needed once zoomed in.
- Sticker, tape, or any other marking object must not block the prints on original package.

See below as an example of these photos.
Photo #1: C2013ABCD01234_F
Photo #2: C2012ABCD01234_F1
Photo #3: C2012ABCD01234_B

Photo #1

Photo #2

Photo #3


LINDT & SPRÜNGLI
MAÎTRE CHOCOLATIER SUISSE DEPUIS 1845
Nutrition Facts
Valeur nutritive
Per 6 squares (33 g) / pour 6 carrés (33 g)
Amount / Teneur
% Daily Value / % valeur quotidienne
Calories / Calories 200
Fat / Lipides 15 g 24 %
Saturated / saturés 11g + Trans / trans 0.1g 53 %
Cholesterol / Cholestérol 5 mg
Sodium / Sodium 40 mg 2 %
Carbohydrate / Glucides 14 g 5 %
Fibre / Fibres 1 g 3 %
Sugars / Sucres 11 g
Protein / Protéines 2 g
Vitamin A / Vitamine A 0 %
Vitamin C / Vitamine C 0 %
Calcium / Calcium 6 %
Iron / Fer 6 %
INGREDIENTS: SUGAR, COCOA BUTTER, MILK INGREDIENTS, COCONUT OIL, COCOA MASS, HAZELNUTS, PALM KERNEL OIL, LACTOSE, PALM OIL, SOYA LECITHIN, BARLEY MALT EXTRACT, ARTIFICIAL FLAVOUR. MAY CONTAIN TRACES OF PEANUTS AND ALMONDS.
INGRÉDIENTS: SUCRE, BEURRE DE CACAO, SUBSTANCES LAITIÈRES, HUILE DE COCO, PÂTE DE CACAO, NOISETTES, HUILE DE PALMISTE, LACTOSE, HUILE DE PALME, LÉCITHINE DE SOJA, EXTRAIT DE MALT D'ORGE, ARÔME ARTIFICIEL. PEUT CONTENIR DES TRACES D'ARACHIDES ET D'AMANDES.
www.lindt.com
C2013ABCD01234
2013_SB567
QUALITY GUARANTEE
Chocoladefabriken Lindt & Sprüngli AG
Kilchberg/Switzerland/Suisse
GARANTIE DE QUALITÉ
Manufactured by / Fabriqué par:
Lindt & Sprüngli AG (Switzerland/Suisse)
Imported by / Importé par:
Lindt & Sprüngli (Canada), Inc.,
Toronto, ON M5H 3M7
18 squares / 18 carrés
STORE IN A COOL AND DRY PLACE
À CONSERVER AU FRAIS ET AU SEC
Best before / Meilleur avant:
30 06 2013
L5742
0 37466 08293 6

Appendix V to Annex A
Sample Storage and Shipping Criteria

Samples will be transported to the Contractor Testing laboratory in accordance with the following standards:

1. All samples must arrive and be tested (including re-test and confirmation test) before the expiry date on the product.
2. Samples that are perishable must be sent by overnight courier.
3. Samples that are shelf stable must be sent by ground unless noted otherwise.
4. Fresh Potato tubers must be transported and stored with minimum light exposure. Results from physically damaged and/or visibly green potato tubers will be rejected from Glycoalkaloids Survey.
5. Samples exceeding the maximum arrival temperature or if the integrity of the sample or its packaging has been compromised, must be re-sampled by the Contractor.

Storage and transportation of the laboratory samples must be carried out in conditions that help avoid any changes in the product. The instructions described below must be followed:

1. Deliver samples to the laboratory within 2 weeks for non-perishable samples.
2. For perishable samples, cool samples rapidly at a temperature between 0 and 5^{o} Celsius prior to shipping. If perishable samples are not shipped immediately, they should be stored in a refrigerator.
3. Perishable samples must be transported with suitable refrigerant capable of maintaining the samples at a temperature between 0 and 7^{o} Celsius.
4. Refrigerated samples must be transported in insulated shipping containers of rigid construction so that they will arrive at the laboratory in good condition.
5. The size of the shipping container should be sufficient to hold the samples.
6. Shipping containers, refrigerant and packing materials are to be clean, dry and sanitary.
7. Samples should be packed tightly to prevent shifting within the shipping container but not too tightly as to compress or damage the samples during transport. Use scrunched up newspaper, shredded paper, Styrofoam nuggets, or other suitable material.
8. Do not freeze refrigerated products.

Willingdon Green Food Composition Laboratory Methods Manual
BFCL-002 Document Control #__1__

Aflatoxins in Food Products - Immunoaffinity Column Method (Based on AOAC 990.33 and AOAC 991.31(2000))

This method is carried out according to the procedures outlined in AOAC 990.33 and AOAC 991.31 (2000) using immunoaffinity column cleanup (AOAC 991.31 Section E - Preparation and extraction of samples, and Section F - Affinity column chromatography), and an HPLC with fluorescence detector (AOAC 990.33 section H - Derivatization, and section I - LC Determination), and post-column derivatization (Journal of Chromatography, 367 (1986), 231-236).

*Scope and Application

This method is applicable to nut and nut products, corn or corn cereal products, dates and dried fruit products.

*Principle

Samples are extracted with NaCl and a methanol/water solution. The filtrates are cleaned up with immuno-affinity columns, followed by derivatization procedure, before being analysed by HPLC with fluorescence detector.

Important Notes

- Aflatoxins are classified as Group 1 carcinogens, a classification restricted to those proven to cause cancer in humans. Take particular precautions in preparation and analysis of samples.
- Mycotoxin contamination of particulate products, such as nuts, is likely to occur in “pockets of high concentration” which may not be randomly distributed. Therefore, the total laboratory sample must be included in the sample preparation process.
- For samples consisting of nuts in the shell, all analytical results must be calculated and reported on the basis of the nut meat portion only.
- Work with samples and standards with ceiling lights off, and use hoods which are equipped with low UV lights.
- This method describes two derivatization procedures. Either procedures can be used, though post-column procedure is preferred.

Method Description

I) Apparatus

HPLC: Waters 6000A; Waters Multi λ 2475 Scanning Fluorescence detector, or equivalent
Column: Supelcosil LC-18, 25 cm x 4.6 mm, or equivalent
Guard column: Waters C18 Novakpak or equivalent

Affinity column: Aflatest P column (Vicam, 29 Mystic Ave, Somerville, MA 02145).

*II) Materials and Reagents

Methanol, HPLC grade
Acetonitrile (ACN), HPLC grade
Water (MQ, deionized, HPLC grade)
Sodium Chloride
Filter paper Whatman No 1
Glass microfibre paper
Potassium Bromide
Nitric Acid
AflaTest mycotoxin testing system (VICAM immunoaffinity columns, part Number 1682D) or equivalent
0.45 µm PTFE filter disks

*Preparation of Standard Solutions and Spikes

Prepare individual aflatoxin stock solutions (approximate 10 µg/mL) in acetonitrile. Determine concentration by UV absorbance as described in AOAC 971.22, using molar absorptivities (*e*) of 20700 for Aflatoxin B_1, 22500 for B_2, 17600 for G_1, and 18900 for G_2. Use the molecular weight of 312.3 for Aflatoxin B_1, 314.3 for B_2, 328.3 for G_1, and 330.3 for G_2. Standard stock solutions have an expiry date of 12 months when stored at -20˚C.

Calibrate UV-Vis spectrometer as per SOP *64uvvis* prior to determining concentrations of standard stock solutions. Determine the exact concentration by maximum UV absorbance at wavelength close to 350 nm using the following equation:

$$C_{stock}, \mu g/mL = A_{max} \times MW \times 1000 / e$$

C_{stock}, µg/mL of Aflatoxin $B_1 = A_{max} \times 15.09$
C_{stock}, µg/mL of Aflatoxin $B_2 = A_{max} \times 13.97$
C_{stock}, µg/mL of Aflatoxin $G_1 = A_{max} \times 18.65$
C_{stock}, µg/mL of Aflatoxin $G_2 = A_{max} \times 17.48$

Transfer appropriate quantity of individual aflatoxin stock solutions to a volumetric flask and bring solution to the mark with acetonitrile to obtain a mixed aflatoxin stock solution containing 0.25 µg/mL of aflatoxins B_1 and G_1 and 0.125 µg/mL of aflatoxins B_2 and G_2. Mixed stock solutions have an expiry date of 3 months when stored at -20˚C.

Volume in mL of B_1, G_1 to be diluted = (0.25 µg/mL * 10 mL) / C_{stock}
Volume in mL of B_2, G_2 to be diluted = (0.125 µg/mL * 10 mL) / C_{stock}

To make up working standard solution:

Make up 100 µL of mixed working standard to a final volume of 10 mL in 10% of acetonitrile in water after derivatization (for pre-column procedure), or without derivatization (for post-column procedure), giving HPLC peak areas equivalent to 5

ppb aflatoxins B_1 and G_1 and 2.5 ppb B_2 and G_2 in 50 g of sample. For other concentration levels, proportion appropriately.

To prepare a spike of 5 ppb for aflatoxin B_1 and G_1 and 2.5 ppb for B_2 and G_2 use 1 mL of the stock standard spiked into 50.0 g of blank matrix (plus 10 g of NaCl). For other fortification levels, proportion appropriately.

For dried fruits (eg dates), to prepare a spike of 5 ppb for aflatoxin B_1 and G_1 and 2.5 ppb for B_2 and G_2 use 1 mL of the stock standard spiked into 100.0 g of blank dates/water slurry (plus 10 g of NaCl). The slurry contains 50.0 g of dried fruit matrix and 50.0 mL of water. For other fortification levels, proportion appropriately.

*III) Procedure

a) Sub-Sampling

Samples are submitted by CFIA food inspectors, according to CFIA Food Safety Investigation Program work specifications. If necessary, samples are ground with Retsch mill or Hobart grinder, then homogenized with Hobart mixers. A sub-sample of about 500 g is taken and stored in the walk-in fridge.

Dates samples should be ground up with the Hobart grinder and then soaked in water overnight so that the samples soften to allow for easy mixing and homogenization. The amount of water used should be equivalent to the amount of sample. For example, 1 kg of dates should be ground up and soaked in 1 L of water. The dates/water mix can now be homogenized with the Hobart mixer, creating a slurry. A sub-sample of about 1000 g is taken and stored in the walk-in fridge.

b) Extraction of Samples

Weigh 50.0 g sample and 10 g NaCl into 1 litre blender jar. Add 250 mL of 60% Methanol (in water) to jar, cover with lid and blend at high speed (high enough to obtain very thorough mixing without splashing) for 3 minutes. Filter through Whatman No.1 paper or equivalent. Centrifuging may be helpful if separation is slow.

For dates, weigh 100.0 g of homogenized dates slurry and 10 g of NaCl into 1 litre blender jar. Add 200 mL of 75% Methanol (in water) to jar, cover with lid and blend at high speed (high enough to obtain very thorough mixing without splashing) for 3 minutes. Filter through Whatman No.1 paper or equivalent. Centrifuging may be helpful if separation is slow. At this point, the amount of sample used, the proportion of solvent and water are the same as that for the other commodities. The rest of the procedure for dates samples is the same as that for the other commodities.

Pour 20 mL filtered extract into a clean vessel. Add 20 mL Milli-Q water and mix well.

Filter dilute extract through glass microfibre paper or equivalent immediately before affinity column chromatography. FILTRATE SHOULD BE CLEAR.

c) Affinity Column Chromatography

Record lot number of AflaTest columns in the worksheet. If aflatoxin analysis hasn't been performed for two or more months, the immunocolumns must be checked by running a standard in reagent blank through a column prior to analysis of samples. Recoveries must be satisfactory (e.g., meet the requirements in the method for a spike sample).

Connect an Aflatest column to a vacuum manifold. Pipet out the buffer solution in the column and attach the barrel of a 20 mL disposable syringe to the top of the column.

Pipet 10 mL of filtered extract into 20 mL syringe barrel, and pass through the column at flow rate of ca 3 mL/min (ca. one drop/sec), using vacuum to control the flow rate. Wash the column with 20 mL MQ water at 3 mL/min. Disconnect the vacuum and remove the column from the manifold. Use kimwipes and swabs to remove any excess water in column inlet and outlet.

Slowly elute aflatoxins from the column by passing 2 x 1.0 mL acetonitrile through the column. The acetonitrile should take ca 1-2 minute to pass through the column; control the flow rate with a 20 mL syringe. Collect the eluant in a 15 mL graduated test tube.

d) Derivatization

i) Pre-Column Derivatization

EVAPORATE TO DRYNESS under N_2 stream at ca. 45˚C. Minimize time residue is left dry. Add 200 µL hexane and 60 µL trifluoroacetic acid to dry residue in vial, and vortex for 30 seconds. Let mixture sit 5 min; then add 1.94 mL H_2O : ACN (90 :10) to the vial and vortex for 30 seconds. Allow layers to separate for at least 10 min. The lower aqueous phase is used for HPLC analysis.

The same derivatization procedure is also carried out for standards except that 10 mL of aqueous solution is used.

ii) Post Column Derivatization

Evaporate to about 0.2 to 0.5 ml under N_2 stream at ca. 45°C, then add water to a final volume of 2.0 ml. Vortex well. Transfer samples to HPLC glass vials by filtering them through 0.45 µm PTFE filter disks to ensure samples are free of particulate matter.

Run standard in duplicate prior to injection of samples. Inject standard again after all samples have been run.

***Local Specifications**

a) Post-Column Derivatization

To install Kobra cell on line, follow the R-Biopharm Rhône's installation instruction.

To prepare mobile phase for use with post column derivatization, add to 1 L of H_2O, 119 mg of potassium bromide and 350 µl of 4M nitric acid. Mix well.

Recommended HPLC conditions Mobile phase: $H_2O/CH_3OH/CH_3CN$ (64/18/18) Flow rate: 1.0 - 1.2 mL/min Approx. pressure: 2000 psi Column: Supelcosil LC-18 (4.6 mm x 25 cm) or equivalent Approx. pressure: 2000 psi Guard column: Waters C18 or equivalent Kobra cell: cell current 20 µA Injection volume: 50 uL Run time: 25 min *Approximate retention times* Aflatoxin G2 Rt: 12 min Aflatoxin G1 Rt: 14 min Aflatoxin B2 Rt: 17 min Aflatoxin B1 Rt: 21 min	*Fluorescence Detector conditions* Excitation: 364 nm Emission: 434 nm Gain: x 1 Filter: 4.0 sec *Performance* Separation: baseline Repeatability: CV: 36% for B1 at 1 ppb 27% for B2 at 0.5 ppb 17% for G1 at 1 ppb 23% for G2 at 0.5 ppb Recovery: 70 -110%, average of 4 aflatoxins (for G2, recovery >50%). Detection limit: 0.5 -1 ppb Reporting limit:1 ppb for each aflatoxin

b) Pre-column Derivatization

Recommended HPLC conditions Mobile phase: $H_2O/CH_3OH/CH_3CN$ (64/18/18) Flow rate: 1.0 - 1.2 mL/min Approx. pressure: 2000 psi Column: Supelcosil LC-18 (4.6 mm x 25 cm) or equivalent Approx. pressure: 2000 psi Guard column: Waters C18 or equivalent Injection volume: 50 uL Run time: 25 min *Approximate retention times* Aflatoxin G1 Rt: 12 min Aflatoxin B1 Rt: 14 min Aflatoxin G2 Rt: 17 min Aflatoxin B2 Rt: 21 min	*Fluorescence Detector conditions* Excitation: 364 nm Emission: 434 nm Gain: x 1 Filter: 4.0 sec *Performance* Separation: baseline Repeatability: CV: 36% for B1 at 1 ppb 27% for B2 at 0.5 ppb 17% for G1 at 1 ppb 23% for G2 at 0.5 ppb Recovery: 70 -110%, average of 4 aflatoxins (for G2, recovery >50%). Detection limit: 0.5 -1 ppb Reporting limit:1 ppb for each aflatoxin

Uncertainty: 13.3 ppb at a level of Total aflatoxin of 60.2 ppb.

The data used to estimate the MU for this method is from a positive control sample previously found to contain aflatoxin B1 and B2. It is run with every batch of test samples. Relative standard deviations [RSDs] for the results of aflatoxin B1 and B2 are calculated. Since the sample does not contain G1 or G2 aflatoxins, the RSDs for these two are approximated from the RSDs of aflatoxins B1 and B2. Standard deviations at specified levels are calculated for each toxin, and combined to give an overall MU estimate.

Expanded Uncertainty: 0.6 ppb at a level of aflatoxin B1 of 5.2 ppb.

The data used to estimate the MU for this method is from a NIST standard reference material found to contain aflatoxin B1, B2 and G1. It is run with every batch of test samples. Relative standard deviations [RSDs] for the results of aflatoxin B1 and B2 are calculated. Only aflatoxin B1 is documented here mainly because it is the major component of most real samples.

***Worksheet Details**

Template	RDIMS document #585337
Title	Aflatoxin YYMMDDinit, where init are the initials of the analyst
Organisation	RAU6A
File Number	35 35201
Document Type	TECH
Notes / Tracking No.	worksheet-chem
Security Access	Burnaby Lab All Staff - Custom (Deselect 'Edit Profile') Burnaby Lab QAOs - full access

***Method Implementation**

A one point or multi-point calibration is used. In the former case, samples are diluted to produce a detector response of 50 - 150% that of the single point standard. A sample spike, using a commodity being tested or an aflatoxin free control sample, and a QC sample, using a commodity previously testing positive for aflatoxin, are done with every batch.

If total aflatoxin found is > 8 ppb, repeat analysis on two additional replicates and report average value of three analyses. If total aflatoxin found is >15 ppb, run an additional un-derivatized sample as a confirmation to see if the concentration of G1 and B1 is greatly reduced, or discuss with the SM.

Samples of Iranian pistachios are analysed in triplicate.

Critical Control Points

Control Point	Acceptable Control
Light sensitivity of aflatoxin	Use low UV light only during analysis
Evaporating the solvent (for pre-column derivatization procedure only)	Evaporate to dry under N_2 stream at ca. 45˚C. Minimize time residue is left dry.

***Reporting**

Report a separate analytical result for each of the four individual aflatoxins. Total aflatoxin

must also be reported for each specimen. The “total aflatoxin” result is determined as the sum of all positive individual aflatoxin results.

Results are to be interpreted as follows:

- Total aflatoxin below detection limit is reported as ‘Not Detected'. Enter ‘<0' in the result entry field to have LSTS automatically insert the phrase ‘Not Detected at the Reporting Limit’ in the Report of Analysis.
- Total aflatoxin less than or equal to 15 ppb – Satisfactory
- Total aflatoxin between 15-23 ppb – Investigative
- Total aflatoxin greater than 23 ppb – Unsatisfactory, in violation of Food and Drug Regulations B.01.046(1)(n)

For proficiency test (PT) schemes, report results according to instructions received with PT samples.

Nut Meat Percentage by Weight for Nuts in the Shell	
Type of Nut	**Nut Meat % by Weight**
Peanuts	70
Pistachios	50
Brazil Nuts	50

(Values from Health Protection Branch Food Directorate (now Health Canada) work specifications; 1996 method HPB-FC-14).

Signature

Science Manager Date

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 Procedure SOP-DAR-LAB-002.

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. Verification studies were also performed on multi-grain cereal, rice beverage, pear-based candy, and seaweed. 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).

3.2 This method has been used to determine concentrations of six arsenic species in infant rice cereal ranging from 0.39 ng/g to 1500 ng/g, and higher levels with suitable dilution; pear-based pureed baby food ranging from 0.23 ng/g to 750 ng/g, and higher levels with suitable dilution and in water ranging from 0.043 ng/mL to 100 ng/mL, and higher levels

with suitable dilution. For analytical ranges and LODs/LOQs of individual species, consult Appendix 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 different 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 - Defines 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 Food processor, Cuisinart, Robot coupe Blixer BX64 or equivalent

5.1.2 Balance suitable for ± 0.01 g and ± 0.0001 g ranges

5.1.3 Adjustable volume pipetters, various sizes

5.1.4 Pasteur pipets or plastic transfer pipets

5.1.5 50 mL disposable plastic tubes and caps, Digitubes or equivalent. Rinse with de-ionized water and allow to dry prior to use

5.1.6 Centrifuge tubes and micro-centrifuge tubes

5.1.7 14 mL disposable plastic tubes, Falcon tubes or equivalent

5.1.8 Vortex mixer

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

5.1.10 13 or 25 mm x 0.20 µm Nylon syringe filters or equivalent

5.1.11 Luer Lock syringes

5.1.12 0.20 µm Nylon vacuum filters, Phenomenex or equivalent

5.1.13 0.6 mL plastic autosampler vials, or equivalent, and snap caps

5.1.14 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.

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

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

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

5.1.16 Teflon bottles – various volumes

5.1.17 Stirring plate with stir bars

5.1.18 pH Meter with calibrating solutions

5.1.19 Adjustable volume dispenser (capable of delivering a volume of 15 mL)

5.1.20 Teflon squeeze bottle

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

5.1.22 Centrifuge, Beckmann Coulter Allegra X-15R or equivalent, capable of spinning at a maximum of 4750 rpm (5250g)

5.1.23 Micro-centrifuge, VWR Galaxy 16D or equivalent, capable of spinning at a maximum of 14000 rpm (16000g)

5.1.24 Teflon weigh spatulas

5.1.25 Rack capable of holding 24 digestion vessels

5.1.26 Spex 6970EFM Cryomill and associated accessories, or equivalent

5.1.27 Volumetric flasks – various volumes

5.2 REAGENTS

5.2.1 Deionized water (DIW), 18.0 MΩ•cm or better

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

5.1.4.1 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.

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

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

5.2.10 Reference Material: NIST 1568a Rice Flour Standard Reference Material, or equivalent containing a certified value for total arsenic.

5.2.11 Reference Material: BCR-279 Sea Lettuce Standard Reference Material, or equivalent containing a certified value for total arsenic.

5.2.12 Primary Standards

NOTE: Stability studies have shown that the Arsenic speciation standards are stable for one year from preparation date.

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.

5.2.12.3.1 2000 µg/mL AsC solution (nominal value): Weigh 0.0200 g of AsC 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. Transfer the solution to a 14 mL Falcon tube. Note the exact weight and subsequent concentration. Refrigerate.

5.2.12.4 Arsenobetaine (AsB), Fluka Chemicals, purum p.a.; > 95.0%, Catalogue No. 11093 or equivalent. Store at room temperature.

5.2.12.4.1 500 µg/mL AsB solution (nominal value): 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. Transfer the solution to a 14 mL Falcon tube. Note the exact weight and subsequent concentration. Refrigerate.

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

5.2.12.5.1 2000 µg/mL MMA solution (nominal value): 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. Transfer the solution to a 14 mL Falcon tube. Note the exact weight and subsequent concentration. Refrigerate.

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

5.2.12.6.1 1000 µg/mL DMA solution (nominal value): Weighing 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. Transfer the solution to a 14 mL Falcon tube. Note the exact weight and subsequent concentration. Refrigerate.

5.2.13 Intermediate Standards

NOTE: Stability studies have shown that the Arsenic speciation standards are stable for one year from preparation date. Intermediate standard solutions are refrigerated.

5.2.13.1 As^{+3} / DMA Intermediate Standard Solution: Transfer the volume of primary solutions as indicated in Table 1 to a single 25 mL volumetric flask. Make to volume with 25% MeOH v/v and invert to mix. Transfer the solution to a 50 mL Digitube.

Table 1: Guide for preparation of the As^{+3}/DMA intermediate standard solution

As Species	Stock Concentration (µg/mL) (Nominal Value)	Volume of Primary Standard Solution used (mL)	Intermediate Standard Solution Concentration (µg/mL) (Nominal Value)
As^{+3}	1000	0.5	20
DMA	1000	0.25	10

NOTE: This solution is prepared based on the concentrations of the different arsenic species obtained during a survey of rice-based and pear-based foods when analyzed by LC-ICP-MS. The concentrations of the arsenic species found in Table 1 are not necessarily in arsenic equivalents and do not account for standard purity.

5.2.13.2 As^{+5}/AsC/AsB Intermediate Standard Solution: Transfer the volume of primary solutions as indicated in Table 2 to a single 25 mL volumetric flask. Make to volume with 25% MeOH v/v and invert to mix. Transfer the solution to a 50 mL Digitube.

Table 2: Guide for preparation of the As^{+5}/AsC/AsB intermediate standard solution

As Species	Stock Concentration (µg/mL) (Nominal Value)	Volume of Primary Standard Solution used (mL)	Intermediate Standard Solution Concentration (µg/mL) (Nominal Value)
As^{+5}	1000	0.35	14
AsC	2000	0.25	20
AsB	500	0.50	10

NOTE: This solution is prepared based on the concentrations of the different arsenic species obtained during a survey of rice-based and pear-based foods when analyzed by LC-ICP-MS. The concentrations of the arsenic species found in Table 2 are not necessarily in arsenic equivalents and do not account for standard purity.

5.2.13.3 MMA Intermediate Standard Solution: Transfer the volume of primary solutions as indicated in Table 3 to a single 25 mL volumetric flask. Make to volume with 25% MeOH v/v and invert to mix. Transfer the solution to a 50 mL Digitube.

Table 3: Guide for preparation of the MMA intermediate standard solution

As Species	Stock Concentration (µg/mL) (Nominal Value)	Volume of Primary Standard Solution used (mL)	Intermediate Standard Solution Concentration (µg/mL) (Nominal Value)
MMA	2000	0.35	28

NOTE: This solution is prepared based on the concentrations of the different arsenic species obtained during a survey of rice-based and pear-based foods when analyzed by LC-ICP-MS. The concentrations of the arsenic species found in Table 3 are not necessarily in arsenic equivalents.

5.2.14 Working Standards

NOTE: Stability studies have shown that the Arsenic speciation standards are stable for one year from preparation date. Working standard solutions are refrigerated.

5.2.14.1 High Level As^{+3}/DMA Working Standard: Transfer the volume of As^{+3}/DMA intermediate solution as indicated in Table 4 to a single 25 mL volumetric flask. Make to volume with 25% MeOH v/v and invert to mix. Transfer the solution to a 50 mL Digitube.

Table 4: Guide for preparation of the High Level As^{+3}/DMA Working Standard

As Species	As^{+3}/DMA Intermediate Standard Concentration (µg/mL) (Nominal Value)	Volume of As^{+3}/DMA Intermediate Standard used (mL)	High Level Working Standard Solution Concentration (µg/mL) (Nominal Value)
As^{+3} / DMA	20 / 10 *	2.5	2.0 / 1.0*

* respectively

NOTE: This solution is prepared based on the concentrations of the different arsenic species obtained during a survey of rice-based and pear-based foods when analyzed by LC-ICP-MS. The concentrations of the arsenic species found in Table 4 are not necessarily in arsenic equivalents and do not account for standard purity.

5.2.14.2 Low Level As^{+3}/DMA Working Standard: Transfer the volume of High Level As^{+3}/DMA Working Standard solution as indicated in Table 5 to a single 25 mL volumetric flask. Make to volume with 25% MeOH v/v. Invert to mix. Transfer the solution to a 50 mL Digitube.

Table 5: Guide for preparation of the Low Level As^{+3}/DMA Working Standard

As Species	High Level As^{+3}/DMA Working Standard Concentration (µg/mL) (Nominal Value)	Volume of High Level As^{+3}/DMA Working Standard Concentration used (mL)	Low Level Working Standard solution Concentration (ng/mL) (Nominal Value)
As^{+3} /DMA	2.0 / 1.0 *	2.5	200 / 100 *

* respectively

NOTE: This solution is prepared based on the concentrations of the different arsenic species obtained during a survey of rice-based and pear-based foods when analyzed by LC-ICP-MS. The concentrations of the arsenic species found in Table 5 are not necessarily in arsenic equivalents and do not account for standard purity.

5.2.14.3 As^{+5}/AsC/AsB Working Standard: Transfer the volume of the As^{+5}/AsC/AsB Intermediate Standard solution as indicated in Table 6 to a single 25 mL volumetric flask. Make to volume with 25% MeOH v/v. Invert to mix. Transfer the solution to a 50 mL Digitube.

Table 6: Guide for preparation of the As^{+5}/AsC/AsB intermediate standard solution

As Species	AsC/AsB/As^{+5} Intermediate Standard Concentration (µg/mL) (Nominal Value)	Volume of AsC/AsB/As^{+5} Intermediate Standard used (mL)	Working Standard Concentration (ng/mL) (Nominal Value)
As^{+5} / AsC / AsB	14 / 20 / 10 *	0.25	140 / 200 / 100 *

* respectively

NOTE: This solution is prepared based on the concentrations of the different arsenic species obtained during a survey of rice-based and pear-based foods when analyzed by LC-ICP-MS. The concentrations of the arsenic species found in Table 6 are not necessarily in arsenic equivalents and do not account for standard purity.

5.2.14.4 MMA Working Standard: Transfer the volume of the MMA Intermediate Standard Solution as indicated in Table 7 to a single 50 mL volumetric flask. Make to volume with 25% MeOH v/v. Invert to mix. Transfer the solution to a 50 mL Digitube.

Table 7: Guide for preparation of the MMA Working standard solution

As Species	MMA Intermediate Standard Concentration (µg/mL) (Nominal Value)	Volume of MMA Intermediate Standard used (mL)	Working Standard Concentration (ng/mL) (Nominal Value)
MMA	28	1.24	694.4

NOTE: This solution is prepared based on the concentrations of the different arsenic species obtained during a survey of rice-based and pear-based foods when analyzed by LC-ICP-MS. The concentrations of the arsenic species found in Table 7 are not necessarily in arsenic equivalents and do not account for standard purity.

5.2.15 General Spiking Solution: Transfer the volume of primary solutions as indicated in Table 8 to a single 25 mL volumetric flask. Make to volume with 25% MeOH v/v and invert to mix. Transfer the solution to a 50 mL Digitube.
NOTE: Stability studies have shown that the Arsenic speciation standards are stable for one year from preparation date. The general spiking solution is refrigerated.

Table 8: Guide for preparation of the General Spiking Solution

As Species	Stock Concentration (µg/mL) (Nominal Value)	Volume of Primary Standard Solution used (mL)	General Spiking Solution Concentration (µg/mL) (Nominal Value)
As^{+3}	1000	0.50	20.0
AsC	2000	0.0605	4.84
AsB	500	0.16	3.20
As^{+5}	1000	0.06	2.40
DMA	1000	0.50	20.0
MMA	2000	0.23	18.4

NOTE: This solution is prepared based on the concentrations of the different arsenic species obtained during a survey of rice-based and pear-based foods when analyzed by LC-ICP-MS. This solution is used for rice-based products, fruit-based products, single and multi-grain cereal, and seaweed. The concentrations of the arsenic species found in Table 8 are not necessarily in arsenic equivalents and do not account for standard purity.

5.2.16 Water Spiking Solution: Transfer the volume of primary solutions as indicated in Table 9 to a single 25 mL volumetric flask. Make to volume with 25% MeOH v/v and invert to mix. Transfer the solution to a 50 mL Digitube.
NOTE: Stability studies have shown that the Arsenic speciation standards are stable for one year from preparation date. The water spiking solution is refrigerated.

Table 9: Guide for preparation of the Water Spiking Solution

As Species	Primary Standard Stock Concentration (µg/mL) (Nominal Value)	Volume of Primary Standard Solution used (mL)	Water Spiking Solution Concentration (µg/mL) (Nominal Value)
As^{+3}	1000	0.250	10.0
AsC	2000	0.025	2.0
AsB	500	0.050	1.0
As^{+5}	1000	0.025	1.0
DMA	1000	0.125	5.0
MMA	2000	0.100	8.0

NOTE: This solution is prepared based on the concentrations of the different arsenic species obtained during a survey of water samples when analyzed by LC-ICP-MS. This solution is used for spiking water. The concentrations of the arsenic species found in Table 9 are not necessarily in arsenic equivalents and do not account for standard purity.

5.2.17 LC Mobile Phases (All matrices other than seaweed)

5.2.17.1 10 mM Ammonium Carbonate with 2.5 mM Tartaric Acid in 2% Methanol (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.

5.2.17.2 30 mM Ammonium Carbonate with 2.5 mM Tartaric Acid in 2% Methanol(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.

5.2.18 LC Mobile Phases (Seaweed and NIST 1568a (when preparing with seaweeds) only)

5.2.18.1 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.

5.2.18.2 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.

5.2.18.3 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.

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

6.2 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.

6.3 ICP-MS instrumentation has high voltages, high temperatures and high levels of UV radiation present and analysts must avoid contact or exposure to these hazards.

6.4 Always add concentrated acid slowly into water when diluting.

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

7 POLICY

7.1 Disposable vessels shall be used whenever possible.

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 doesn't 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 Extraction Vessel Preparation

8.2.1 Use table 12 as a guide to determine which extraction vessels are required for each matrix. Rinse the extraction vessels as well as their caps three times with DI water and invert to dry. If preparing seaweeds, rinse a second set of Digitubes equivalent to the set of samples (see 8.3.3). If preparing samples in Digitubes, rinse a second set of Centrifuge tubes equivalent to the set of samples (8.3.3). See Table 12 for sample vessel information.

8.3 Enzymatic Digestion (all matrices other than water)

8.3.1 Add protease to a labelled 50 mL Digitube or centrifuge tube (See Table 12 in 8.3.5 for more information). The amount of protease added will depend on the estimated protein content of the sample. This amount is based on method development experiments performed on rice-based and pear-based products. Use Table 10 as a guide to determine the amount of protease to add to the sample. See QA/QC Considerations 9.5 for additional information. (**Note:** The protease contains natural levels of As^{+5}. The samples are blank-corrected for this contribution therefore it is important to try and keep its mass as consistent as possible.)

Table 10: Guide for the amount of protease to add to samples:

Sample Matrix	Amount of protease (mg)
Rice-based Products (includes dry rice products such as infant rice cereal, white rice, brown rice and rice crackers, and rice beverages) Single-grain and Multi-grain Cereals Reference Materials* Seaweed	60 ± 2
Fruit-based Products (includes baby food, juices, candy, dried fruit snacks, and canned fruit)	30 ± 2

NOTE: A reference material will be prepared with every batch of samples so that a separate reagent blank may be required for its analysis (as will be the case if preparing fruit-based products or seaweeds). *The NIST 1568a Reference material is to be prepared when analyzing rice-based products, cereals, fruit-based products and seaweed. The BCR-279 Reference material is to be prepared when analyzing seaweed.

8.3.2 Add approximately 30 mg of alpha-Amylase and 30 mg of Lipase to each sample tube.

8.3.3 Weigh an aliquot of homogenized sample into the labelled 50 mL digitube or centrifuge tube. Use Table 11 as a guide to determine the amount of sample to weigh. See QA/QC Considerations 9.6 for additional information. For each set of samples, include a reagent blank, and a CRM (if available), reference material, or check sample. Plan to spike one sample of a different matrix type in the run; weigh the sample, a replicate, and a third replicate which will be spiked.

Table 11: Guide for sample weight:

Sample Matrix	Weight of sample (g)
Rice-based Products (includes dry rice products such as infant rice cereal, white rice, brown rice and rice crackers, and rice beverages) Single-grain and Multi-grain Cereals	1 ± 0.5000
Seaweed	0.5 ± 0.2000
Reference Materials	0.4 ± 0.2000 (or as specified in the Certificate of Analysis)
Fruit-based Products (includes baby food, juices, candy, dried fruit snacks, and canned fruit)	2 ± 0.5000

8.3.4 Spike sample(s) at this stage using the General Spiking Solution (5.2.15). A typical spike volume is 0.025 mL of the Spiking Solution.

8.3.5 Add Extraction solution (5.2.2.1) to the digitube or centrifuge tube using either a calibrated dispenser or a teflon squeeze bottle. Use Table 12 as a guide to which extraction vessel to use for the preparation of samples as well as a guide to which method should be used for the addition of Extraction Solution. See QA/QC Considerations 9.8 for additional information.

Table 12: Guide to use of sample vessel and Extraction solution addition method:

Matrix	Extraction vessel	Method of Extraction solution addition
Dry Rice-based Products (includes dry rice products such as infant rice cereal, white rice, brown rice, rice crackers and Reference Material)	Centrifuge tube or Digitube	Add 15 mL with a Calibrated Dispenser
Rice Beverages (includes rice milk, rice pudding, and brown rice beverages)	Digitube	Make up to 15 mL using a Teflon Squeeze bottle
Fruit-based Products (includes baby food, juices, and canned fruit)	Digitube	Make up to 15 mL using a Teflon Squeeze bottle
Seaweed	Centrifuge tube or Digitube	Add 15 mL with a Calibrated Dispenser
Fruit-based Products (includes dried fruit and candy snacks)	Centrifuge tube or Digitube	Add 15 mL with a Calibrated Dispenser
Single-grain or Multi-grain Cereal	Centrifuge tube or Digitube	Add 15 mL with a Calibrated Dispenser

8.4 Calibration Curve Preparation: Use Table 13 as a guide to determine which sample matrices require the use of matrix fortified diluent to prepare calibration standards. See QA/QC Considerations 9.7 for additional information.

8.4.1 If a matrix-fortified calibration curve is required, prepare an extra four 50 mL Digitubes or centrifuge tubes (see Table 12 as a guide) by adding the enzymes and the sample as per 8.3.1, 8.3.2, and 8.3.3. (Prepare only one extra 50 mL digitube or centrifuge tube if extracting seaweed). The same sample is weighed in the four digitubes or centrifuge tubes and is chosen to represent the matrices being analyzed. For seaweed, similar types must be extracted together so that the matrix-fortified curve is representative of the samples.
NOTE: Red Seaweed contains a higher amount of agar and carrageenan than other species of seaweed which make it difficult to analyze. Red Seaweed is to be analyzed using a matrix-fortified calibration curve prepared using another type of seaweed.

8.4.2 If a reagent blank matched calibration curve is required, prepare an extra four 50 mL Digitubes or centrifuge tubes (see Table 12 as a guide) by adding the enzymes as per 8.3.1 and 8.3.2. Use Table 13 as a guide to determine which sample matrices

require the use of reagent blank matched diluent to prepare calibration standards. See QA/QC Considerations 9.7 for additional information.

8.4.3 If a reagent blank matched calibration curve is required only for the NIST 1568a reference material (as per Table 13) (all other samples will require a matrix-fortified calibration curve), prepare an extra two 50 mL digitubes or centrifuge tubes (see Table 8 as a guide) by adding the enzymes as per 8.3.1 and 8.3.2. A matrix-fortified calibration curve is required for the BCR-279 reference material. Prepare one extra 50 mL digitube or centrifuge tube containing the BCR-279 reference material (see Table 12 as a guide).

8.4.4 A 25% Methanol (v/v) calibration curve is required for water analysis. Prepare the calibration standards as per Table 14 using 25% (v/v) Methanol (2.5 mL of Methanol made up to 10 mL with DIW).

Table 13: Guide for the choosing of diluent in the preparation of a calibration curve:

Matrix	Calibration Curve Diluent
Fruit-based Products (includes baby food, juices, candy, dried fruit snacks, and canned fruit)	Matrix-fortified diluent (8.4.1)
NIST 1568a Reference Material	Reagent-blank matched diluent (8.4.3)
Rice-based Products (includes dry rice products such as infant rice cereal, white rice, brown rice and rice crackers) Single-grain Cereals	Reagent-blank matched diluent (8.4.2)
Rice Beverages (includes rice milk, rice pudding, and brown rice beverages)	Matrix-fortified diluent (8.4.1)
BCR-279 Reference Material	Matrix-fortified diluent (8.4.3)
Seaweed	Matrix-fortified diluent (8.4.1)
Water	25% (v/v) Methanol diluent (8.4.4)
Multi-grain Cereal	Matrix-fortified diluent (8.4.1)

8.4.5 Seal all of the tubes tightly. Vortex the sample for ten seconds.

8.4.6 Place tubes in the rotating mixer inside the incubator at 37 °C for a minimum of 16 hours.

8.4.7 Matrices other than seaweed: After incubation, transfer the samples to centrifuge or micro-centrifuge tubes (if in Digitubes). Centrifuge the tubes at 3000 rpm (2094 x g) if using the centrifuge or at 14000 rpm (16000 x g) if using the micro-centrifuge for 10 minutes, then pour a 1-2 mL aliquot into a syringe fitted with a 0.20 µm nylon filter. Filter into a 0.6 mL autosampler vial.

8.4.8 Seaweed: After incubation, transfer the samples to centrifuge tubes (if in Digitubes). Centrifuge the tubes at 3000 rpm (2094 x g) for 10 minutes. Transfer the supernatant to a pre-rinsed labelled 50 mL Digitube. Add 15 mL of extraction solution (5.2.2.1) to the original centrifuge tube using a calibrated dispenser. Vortex for 10 seconds and return the samples to the centrifuge. Centrifuge the tubes at 3000 rpm (2094 x g) for 10 minutes. Transfer the supernatant to the same pre-rinsed labelled 50 mL Digitube as before. Once more, add 15 mL of extraction solution to the original centrifuge tube using a calibrated dispenser. Vortex for 10 seconds and return the samples to the centrifuge. Transfer the supernatant to the same pre-rinsed labelled 50 mL Digitube as before. Make up to the 50 mL mark using 25% MeOH (v/v). Invert to homogenize. Pour a 1-2 mL aliquot into a syringe fitted with a 0.20 µm nylon filter. Filter into a 0.6 mL autosampler vial.
NOTE: The NIST 1568a reference material is to be prepared as described in Section 8.3. Only 15 mL total volume of extraction solution is to be added prior to centrifugation and filtration into an autosampler vial.
NOTE: Red Seaweeds will be difficult to filter and may require the use of several filters to obtain enough extract to analyze. These samples should be run one per sequence, at the end of the sequence, following the last calibration check standard. Run several washes after the red seaweed extract.

8.4.9 If preparing a matrix-fortified or reagent blank matched calibration curve for all matrices other than seaweed, transfer the samples to centrifuge tubes (if in digitubes) and place in a centrifuge. Centrifuge the samples for 10 minutes at 3000 rpm (2094 x g). Combine the matrix diluent in a pre-rinsed teflon bottle and shake to homogenize. If preparing a matrix-fortified calibration curve for seaweed, follow the procedure in 8.4 to obtain the 50 mL of matrix diluent. Invert to homogenize. Do not filter at this point.

8.5 Water Preparation

8.5.1 For each set of samples, include a reagent blank, and a CRM (if available), reference material, or in-house check sample. Plan to spike two samples in the run; pipette the samples, a replicate for each sample, and a third replicate for each sample which will be spiked.

8.5.2 Using an adjustable pipetter, place 7.5 mL of water sample into a 14 mL labelled falcon tube.

8.5.3 Spike sample(s) at this stage using the Water Spiking solution (5.2.16). A typical spike volume is 0.025 mL of the Spiking Solution.

8.5.4 Using an adjustable pipetter, place 2.5 mL of methanol into the same 14 mL labelled Falcon tube. Invert to mix. Filter an aliquot into an autosampler vial using a 0.20 µm Nylon syringe filter.

8.6 Calibration Standards Preparation: The following are the suggested instructions for the preparation of calibration standards used in the analysis of rice-based products, fruit-based products, water, seaweed, and single and multi-grain cereal. Higher calibration levels or other methods of calibration may be required when dealing with other food products. See QA/QC Consideration 9.9 for additional information.

8.6.1 Prepare calibration standards using volumes and solutions indicated in Table 14. Transfer the indicated volume of appropriate working standard solution to 14 mL Falcon tubes, add the matrix diluent (see Table 13), cap the tubes and invert to mix. Filter an aliquot of the calibration standards into an autosampler vial using a 0.20 µm Nylon syringe filter. Concentrations of the Arsenic Species in the Calibration Standards are found in Table 1.

Table 14: Guide for the 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: If preparing a calibration curve using the reagent blank matched diluent for the analysis of the NIST 1568a reference material or if preparing a calibration curve using the BCR-279 matrix matched diluent for the analysis of the BCR-279 reference material, only Standard 2 and Standard 4 need to be prepared. The Reagent Blank prepared with the reference material (if different from the reagent blank prepared with the samples) will be used as Standard 0. See Appendix 3 for Example chromatograms of the Arsenic speciation calibration standards.

Table 15: Arsenic Species Concentrations found in the Calibration Standards (Nominal Values)

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

NOTE: The concentrations of the arsenic species found in Table 15 are not necessarily in arsenic equivalents and do not account for standard purity.

8.7 LC-ICP-MS Analysis

8.7.1 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.

8.7.2 Check the ICP-MS performance according to manufacturer's specifications. See Appendix 2 for suggested ICP-MS and LC operating conditions.

8.7.3 Load the autosampler. Use the mobile phases prepared in 5.2.17 for all matrices other than seaweed. Use the mobile phases prepared in 5.2.18 for seaweeds and the NIST 1568a and BCR-279 reference materials. Indicate on the control chart for the NIST 1568a when it was analyzed using the seaweed mobile phases. Initiate analysis as per manufacturer's instructions. Ensure the output from the LC will either calculate or allow for measurement of analyte peak response.

8.8 Calculation and Expression of Results

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

8.8.1.1 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. See Table 11 for the Arsenic Equivalency Factors used to calculate the concentration of the arsenic species.

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

0.543 x 1000 ng/mL = 543 ng/mL

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

Table 16: Equivalency Factors used to calculate Arsenic Concentrations

Arsenic Species	Linear Formula	Arsenic mass/Arsenic Species mass	Standard Purity	Arsenic Equivalency Factor
AsC	$C_5H_{14}AsBrO$	74.9 / 244.99	-	0.306
AsB	$C_5H_{11}AsO_2$	74.9 / 178.06	95 %	0.400
DMA	$(CH_3)_2As(O)OH$	74.9 / 138	98 %	0.532
MMA	CH_3AsO_3•2Na.•$6H_2O$	74.9 / 291.9	97.5%	0.250

NOTE: These equivalency factors are based on current stock compositions. The stock composition can change based on the source of the neat standard.

8.8.1.2 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 mass counts)
m = slope of calibration curve
b = intercept

8.8.2 For all samples except waters, calculate the final concentration (ng/mL) of arsenic equivalents in the sample extract using the equation above 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:

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

8.8.3 For waters, calculate the final concentration (ng/mL) of arsenic equivalents in the sample extract using the equation above 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 below:

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

8.8.4 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 8.8.2 and 8.8.3. Table 17 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 17: RDIMS File Numbers for Data Calculations

Matrix	RDIMS #
Rice-based Products (includes dry rice products such as infant rice cereal, white rice, brown rice and rice crackers) Single-grain Cereals	3158952
Fruit-based Products (includes baby food, juices, candy, dried fruit snacks, and canned fruit) Multi-grain Cereal	3168239
Water	3168272
Seaweed	3277051

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. Table 18 lists the RDIMS numbers associated with the excel spreadsheets used to plot the results for the control sample or certified reference material.

Table 18: RDIMS File Numbers for Control Charts

Matrix	RDIMS #
NIST 1568a Rice Flour	2599166
BCR-279 Sea Lettuce	3005581
Water (Inorganic Arsenic)	3169817

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

9.4 See Appendix 1 for the limit of detection (LOD) and the limit of quantitation (LOQ) for each arsenic species in infant rice cereal, pear-based pureed baby food, and in water as determined during method validation. See Appendix 1 for the limit of detection (LOD) and the limit of quantitation (LOQ) for each arsenic species in seaweed as determined during method verification.

9.5 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.

9.6 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 high moisture content and high expected speciated arsenic concentrations such as is found in rice-based beverages.

9.7 Foods other than rice-based or fruit-based products submitted for analysis must have method development experiments conducted to evaluate possible matrix effects.

9.8 The decision of which method to use for the addition of Extraction solution as well as for the choice of sample vessel will be based on the moisture content of the

sample matrix. It is assumed that a sample with a higher moisture content will have its moisture contribute to the final volume of the extraction solution. Where a final volume of 15 mL is desired, a 1 gram sample of a dry product will not contribute to the extraction volume and so 15 mL of Extraction solution will be added to the sample using a calibrated dispenser. In doing so, either a 50 mL Digitube or a 50 mL Centrifuge tube can be used as the extraction vessel. A 1 gram sample of a moisture-laden product will contribute to the extraction volume and so the sample is made up to a final volume of 15 mL using the markings found on the Digitube.

9.9 If 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.

9.10 (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, juices, jams and sauces) are the same as those determined for pear-based pureed baby food). This method has also been used to determine concentrations of four arsenic species in seaweed. (The analytical range and LODs/LOQs for seaweed can be found in Appendix 1).

Appendix 1

The analytical range, the LOD and the LOQ for each arsenic species are presented in Tables 1 and 2 as determined during method validation for infant rice cereal, pear-based pureed baby food, and water. For each species except As^{+5}, the LOD was calculated as 3 x SD + Mean of the Noise of the matrix. For As^{+5}, in the case of the infant rice cereal and the pear-based pureed baby food, the LOD was calculated as 3 x SD of the mean of the protease concentration in the same matrix samples. For As^{+5}, in the case of water and seaweed , the LOD was calculated as 3 x SD + Mean of the Noise of the matrix. For all species, the LOQ was calculated as 3 x LOD. 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 are the same as those determined for pear-based pureed baby food

Table 1: Analytical Range

Species	Analytical Range in Arsenic Equivalents of Infant Rice Cereal (ng/g)	Analytical Range in Arsenic Equivalents of Pear-Based Pureed Baby Food (ng/g)	Analytical Range in Arsenic Equivalents of Water (ng/mL)
AsC	0.39 - 460	0.52 - 230	0.061 - 3.18
AsB	0.41 - 300	0.23 - 170	0.083 - 2.20
As^{+3}	0.68 - 1500	0.66 - 750	0.078 - 100
DMA	0.70 - 410	0.36 - 240	0.043 - 30.8
MMA	0.98 - 860	0.58 - 435	0.088 - 23.2
As^{+5}	4.80 - 1050	2.67 - 525	0.127 - 7.00

Table 2: Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Species	LOD (ng/g) in Arsenic Equivalents of Infant Rice Cereal	LOQ (ng/g) in Arsenic Equivalents of Infant Rice Cereal	LOD (ng/g) in Arsenic Equivalents of Pear-Based Pureed Baby Food	LOQ (ng/g) in Arsenic Equivalents of Pear-Based Pureed Baby Food	LOD (ng/mL) in Arsenic Equivalent of Water	LOQ (ng/mL) in Arsenic Equivalents of Water
AsC	0.388	1.163	0.525	1.575	0.061	0.184
AsB	0.407	1.220	0.232	0.697	0.083	0.248
As^{+3}	0.681	2.044	0.660	1.981	0.078	0.235
DMA	0.699	2.096	0.363	1.090	0.043	0.128
MMA	0.982	2.946	0.582	1.745	0.088	0.263
As^{+5}	4.804	14.412	2.669	8.006	0.127	0.381

Table 3: Analytical Range and LOD/LOQs for Seaweed

Species	Analytical Range in Arsenic Equivalents of Seaweed (ng/g)	LOD of Seaweed (ng/g)	LOQ of Seaweed (ng/g)
As^{+3}	10 - 10000	10.0	30.0
DMA	5 - 3080	5.0	15.0
MMA	13 - 2315	13.0	40.0
As^{+5}	10 - 700	10.0	30.0

Appendix 2

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	0.4 L/min
Detector Mode	Pulse

Table 2: LC parameters:

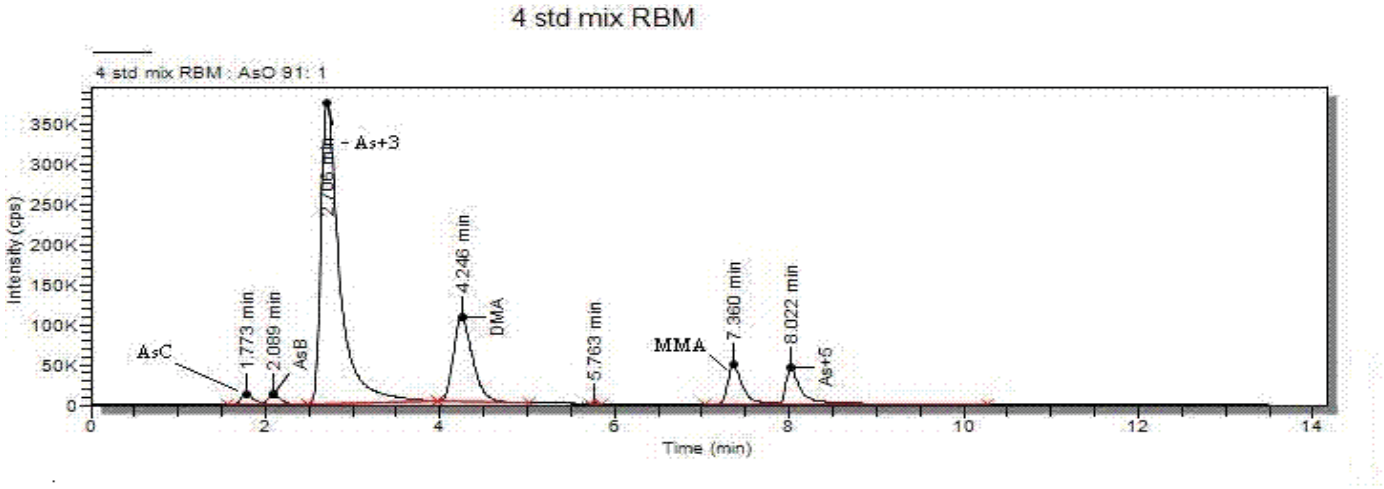
Column	PRP X-100 (250 mm x 4.0 mm x 10 µm), with guard
Pump Setting	gradient (see below)
Flow rate	1.2 mL/min
Run Time	13.5 minutes
Equilibration Time	0.1 minute between runs
Injection Volume	50 µL single injection per vial
Column Temperature	25 º

Table 3: LC 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: LC 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

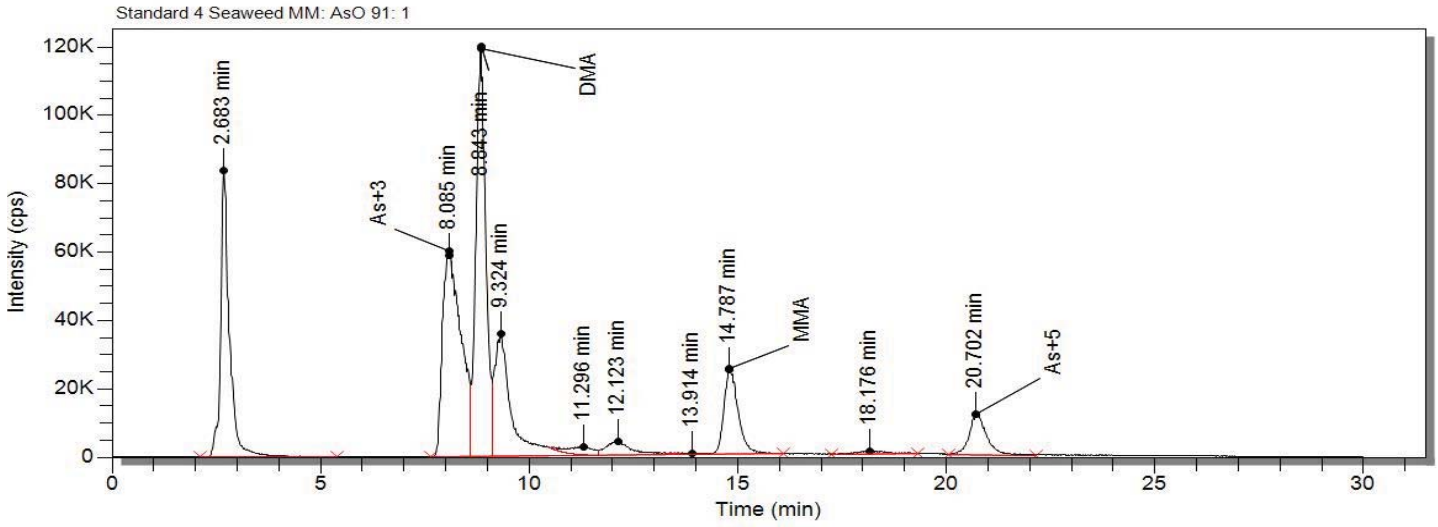


Appendix 3

Example Chromatogram of Arsenic Speciation Calibration Standards using Mobile Phase system for all matrices other than seaweed.

Example Chromatogram of Arsenic Speciation Calibration Standards using Mobile Phase system for seaweed.

Standard 4 Seaweed MM : AsO 91 : 1



Canadian Food Inspection Agency
Ottawa Laboratory (Carling)
Food Chemistry Section

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COUMARIN IN CINNAMON CONTAINING FOOD BY HPLC

1.0 Scope and Field of Application

This method is applicable to the determination of coumarin in various cinnamon matrices such as ground and whole cinnamon bark, cereals, cookies and spice mixes.

The analytical range, reporting limit and other method performance specifications can be found in the validation file for the method.

2.0 Principle and Definitions

There are two types of cinnamon that are sold commercially, Cassia and Ceylon. Coumarin is a naturally occurring compound present in cinnamon, and the concentration varies greatly depending on the cinnamon source. Cassia cinnamon is high in coumarin and typically contains 3000 mg/kg or more. Ceylon cinnamon is very low in coumarin, with results generally less than 100 mg/kg.

A polar solvent is used to extract coumarin and its related constituents from the sample. The compound is separated on an HPLC using a C18 column and is detected at 277 nm with a photodiode array detector (PDA). Coumarin identification is confirmed by comparison of the sample peak spectra to that of the standards and is quantified by comparison with a set of external standards.

3.0 References

3.1 OLC-SOP-021: SOP for Method Familiarization.

3.2 OLC-SOP-012: SOP for Handling and Documenting Non-conformances, Corrective Actions, Complaints and Preventive Actions.

3.3 OLC SOP 023: SOP for the Quality Control of Equipment/Instruments

3.4 OLC SOP 016: SOP for Use of Control Charts

3.5 OLC-FC-265: FLS-2011-001 results template (RDIMS 3136463)

4.0 Reagents and Solutions

4.1 Reagents

4.1.1 Methanol (CH_3OH), EMD Omnisol MX0488-1, or equivalent

4.1.2 Water, de-ionised and Nanopure purified, or equivalent. Used in all instances where water is required.

4.1.3 Phosphoric Acid, 85%, H_3PO_4 EMD HPLC grade PX0996-6 or equivalent.

4.1.4 Acetonitrile (CH_3CN), HPLC grade. Fisher A3396 or equivalent.

4.2 **Solutions**

Note: Solution volumes can be adjusted dependant on the amount required provided the final concentrations remain the same.

4.2.1 **80% Methanol**

Fill a 500mL volumetric flask with 100 mL of water (4.1.2) and complete to volumetric mark with methanol (4.1.1). Solution is used for standard preparation and sample preparation.

4.2.2 **1 mM phosphoric acid**

Pipet 68 µL of phosphoric acid (4.1.3) into a 1 L volumetric flask containing ~700 mL water (4.1.2). Fill to volumetric mark, cap and invert the flask several times to mix.

4.2.3 **Mobile Phase A (60:20:20 1 mM phosphoric acid/ acetonitrile/ methanol)**

Measure 600 mL of 1 mM phosphoric acid solution (4.2.2), 200 mL of acetonitrile (4.1.4) and 200 mL of methanol (4.1.1) in separate graduated cylinders. Pour into a 2 L beaker or glass bottle. Mix the solution and filter on 0.45 µm vacuum filtration system (6.2.2). Mobile phase is prepared fresh prior to analysis.

4.2.4 **Mobile Phase B (90:10 Acetonitrile / water)**

Measure 900 mL of acetonitrile (4.1.4) and 100 mL of water (4.1.2) in separate graduated cylinders. Pour contents into a 2 L beaker or glass bottle. Mix the solution and filter on 0.45 um vacuum filtration system (6.2.2).

5.0 Reference Standards

5.1 Standard

Coumarin, (2*H*-chromen-2-one), Aldrich, C4261, or equivalent. The standard is stored at room temperature.

5.2 Standard Solutions

5.2.1 Stock Standard Solution (1 mg/mL Coumarin)

Weigh approximately 0.1g of coumarin (5.1) to the nearest 0.0001g in a 100 mL amber volumetric flask. Dissolve to volume with 80% methanol (4.2.1). This solution expires after 2 weeks.

5.2.2 Working Standard Solutions

Note that coumarin concentration levels will vary depending on the type of sample. Cassia cinnamon generally contains high levels of coumarin while in Ceylon cinnamon concentrations are generally low. One or both sets of working standards can be prepared depending on the nature of the samples that are being analysed.

Working standards are prepared according to the following tables and diluted to volume with 80% methanol (4.2.1).

High Standards: Use the following set of working standards for Cassia cinnamon products or unknown samples.

Std	Std Stock 5.2.1 (uL)	Final Volume (mL)	Final Conc. (mg/L)
s0	0	10	0
s6	50	10	5
s7	100	10	10
s8	250	10	25
s9	500	10	50
s10	1000	10	100

Low Standards: Use the following set of working standards for Ceylon cinnamon or for samples that fall below the concentration of S6 in the high standards.

Std	Std Stock 5.2.1 (uL)	Final Volume (mL)	Final Conc. (mg/L)
s0	0	50	0
s1	10	50	0.2
s2	20	50	0.4
s3	50	50	1
s4	100	50	2
s5	200	50	4

Working standard solutions are prepared fresh prior to analysis.

6.0 Materials

6.1 Labware

6.1.1 Polypropylene centrifuge tubes, 50 mL Falcon or equivalent.

6.1.2 Electronic pipets, various volumes, with disposable tips, Rainin or equivalent.

6.1.3 Syringes with Luer-Lok tips. Capacity 1mL.

6.1.4 0.45 µm syringe filters, Pall Acrodisc LC13mm PVDF Membrane, 4457T, or equivalent.

6.1.5 HPLC autosampler vials, 2 mL capacity with caps.

6.1.6 0.45 µm vacuum filters, Pall Ultipor N66, NX047100, or equivalent.

6.1.7 Graduated cylinders of various volume

6.1.8 Beakers of various volume

6.1.9 Glass funnels

6.1.10 Volumetric flasks of various volume

6.1.11 Glass bottles of various volume

6.2 **Auxiliary Equipment**

6.2.1 Analytical Balance, capable of weighing to 4 decimal places (0.0001g).

6.2.2 Vacuum filtration apparatus.

6.2.3 Mechanical Shaker, Eberbach 6000, or equivalent.

6.2.4 Spice Grinder, Waring Commercial WSG30, or equivalent.

6.2.5 Centrifuge, Eppendorf Centrifuge 5810, or equivalent.

6.3 **Instrumentation**

6.3.1 **High Performance Liquid Chromatography (HPLC) System**, Waters Alliance 2695 system or equivalent equipped with:

6.3.1.1 Detector :
Waters 996 Photodiode Array or equivalent.
Quantitation wavelength: 277 nm

6.3.1.2 Thermostatic column temperature module or equivalent.
The column temperature is set at 25°C.

6.3.1.3 Column: Luna C18(2) 250mm x 4.6mm (5µm), part # 00G-4252-EO

6.3.1.4 Software: Empower Pro chromatography workstation or equivalent

6.3.2 **HPLC parameters**

Note: These are typical parameters. Individual parameters may be adjusted by the operator.

6.3.2.1 Mobile Phase: See section 4.2.3

6.3.2.2	Flow rate**:**	
	0-20 minutes	100% A at 0.7 ml/min
	25 minutes	100% B at 1.0 mL/min
	45 minutes	100% B at 1.0mL min
	50 minutes	100% A at 0.7 mL/min
6.3.2.3	Inj. volume:	10 µL
6.3.2.4	Run time:	60 min

7.0 Procedure

For every set of ten samples or less, two spiked samples are prepared as per section 7.3 or 7.5. One spiked sample is used for recovery calculations, and the second spiked sample is used to assess repeatability.

Note: For samples with a high amount of coumarin (such as cassia cinnamon) or unknown samples, use sample preparation procedure 7.2 and 7.3. Samples with low levels of coumarin (such as ceylon cinnamon) are prepared using 7.4 and 7.5.

7.1 Homogenization:

Samples are homogenised to get a representative sample for analysis. For samples of cinnamon sticks, the whole sample should be grounded and mixed, as the level of coumarin in each individual stick has been shown to be variable. For processed goods (cereals, tea, cookies etc), only a representative amount of sample is required for grinding and mixing as these samples are homogeneous.

7.1.1 Sample Grinding:

For samples that require grinding, grind sample in the spice grinder (6.2.4) for approximately 30 seconds. The sample may have to be ground in batches and blended together if the entire portion to be ground will not fit in the grinder at once.

7.2 Sample Preparation: high coumarin level and/or unknown samples

7.2.1 Weigh ~ 0.5 g of cinnamon to the nearest 0.0001 g in a 50 mL polypropylene centrifuge tube (6.1.1) and record the weight.

7.2.2 Pipette 50 mL of 80% methanol (4.1.1) into the tube and cap.

7.2.3 Place samples on a mechanical shaker (6.2.3) on “high” setting for

approximately 60 minutes.

7.2.4 Centrifuge samples at approximately 2500 rpm for approximately 10 minutes. Proceed to step 7.6.

7.3 **Fortified Sample: high coumarin level and/or unknown samples**

7.3.1 Weigh ~ 0.5 g of cinnamon to the nearest 0.0001 g in a 50 mL polypropylene falcon tube and record the weight.

7.3.2 Pipette 1 mL of coumarin stock standard (5.2.1) into the tube.

7.3.3 Pipette 50 mL of 80% methanol (4.1.1) into the tube and cap.

7.3.4 Place samples on a mechanical shaker (6.2.3) on "high" settings for approximately 60 minutes.

7.3.5 Centrifuge samples at approximately 2500 rpm for approximately 10 minutes. Proceed to step 7.6.

7.4 **Sample Preparation: Samples with low coumarin level**

7.4.1 Weigh ~ 2 g of cinnamon to the nearest 0.0001 g in a 50 mL polypropylene falcon tube and record the weight.

7.4.2 Pipette 25 mL of 80% methanol (4.1.1) into the tube and cap.

7.4.3 Place samples on a mechanical shaker (6.2.3) on "high" settings for approximately 60 minutes.

7.4.4 Centrifuge samples at approximately 2500 rpm for approximately 10 minutes. Proceed to step 7.6.

7.5 **Fortified Sample: Samples with low coumarin level**

7.5.1 Weigh ~ 2 g of cinnamon to the nearest 0.0001 g in a 50 mL polypropylene falcon tube and record the weight.

7.5.2 Pipette 50 µL of coumarin stock standard (5.2.1) to the tube.

7.5.3 Pipette 25 mL of 80% methanol (4.1.1) into the tube and cap.

7.5.4 Place samples on a mechanical shaker (6.2.3) on "high" settings for approximately 60 minutes.

7.5.5 Centrifuge samples at approximately 2500 rpm for approximately 10 minutes. Proceed to step 7.6.

7.6 **HPLC Analysis**

Filter the diluted samples (7.2 or 7.4), spiked samples (7.3 or 7.5), and working standards (5.2.2) through syringe filters (6.1.4) directly into autosampler vials (6.1.5), and inject on the HPLC system.

Note: Some samples may have a concentration that lies in between the two standard curves. In such cases, the sample can be diluted in order to fall within the low concentration curve.

8.0 Preparation of standard curve.

Quantitation is done by external standard method. Using the working standard solutions (section 5.2.2), prepare a standard curve, ploting peak area versus concentration.

Calculate the slope and intercept of the line using the equation:

$$y = mx + b$$

y = peak area
m = slope
x = concentration
b = y intercept

The correlation coefficient (r), must be greater than 0.995.

9.0 Calculations

9.1 **Concentration in sample**

To determine the concentration of coumarin in the samples (C), interpolate from the standard curves:

$$C = \left(\frac{y - b}{m}\right) \times \frac{V_F}{w_{SA}}$$

Where: C = concentration of coumarin in the sample, mg/kg
y = area count
b = intercept of the standard curve
m = slope of the standard curve
V_F = dilution volume (L)
w_{SA} = sample weight (kg)

9.2 **Calculation of Spike Recovery**

Percent recovery is determined by calculating the theoretical and actual spike concentrations as follows:

9.2.1 **Theoretical Spike concentration**:

$$C_{th} = \frac{c_{ss} \times V_{ss}}{V_f}$$

Where: C (th) = Theoretical concentration of the spike (mg/L)
c (ss) = standard stock (5.2.1) conc. (mg/L)
V (ss) = Volume of spiking solution added (mL)
V (f) = Final Volume (mL)

9.2.2 **Experimental Spike concentration:**

$$C_{xp} = \frac{\left(A_{sp} - A_{sa}\right) - b}{m}$$

Where: C (xp) = Experimental concentration of the spike (mg/L)
A (sp) = Area count of spiked sample
A (sa) = Area count of sample
b = intercept of the standard curve
m = slope of the standard curve

9.2.3 **Recovery Calculation:**

$$\%\text{Recovery} = \frac{C_{xp}}{C_{th}} \times 100\%$$

10.0 Expression of results

10.1 Results are reported in mg/kg.

11.0 Quality Control Plan

11.1 Performance Specifications

Performance specifications can be found in the validation file of the method.

11.2 Critical Control Point

10.2.1 Cinnamon sticks: need to grind the whole package.

11.3 Familiarization

The analyst demonstrates their ability to perform the method by analyzing a sample spiked at four different levels and one at the same level for repeatability, and repeating this set on two days, with acceptable recoveries, as determined by the unit supervisor.

11.4 Quality Control

If either of the control points 10.4.1 or 10.4.2 fail, appropriate corrective action measures are taken as outlined on the control charts for the method. Note that there are two sets of control charts. The control chart to use is dependant on which set of standards and sample preparation were used (high or low).

11.4.1 Spiked Sample:

The recovery for the spiked sample is plotted on the control chart. This value should lie within the control limit as defined on the control charts. Control limits for the recoveries are ± 3 SD from the average recovery.

11.4.2 Repeat Samples:

The absolute difference of the repeat measurements is plotted on the control chart. This value should lie within the control limit as defined on the chart. The control limit for the absolute difference is 3SD from zero.

12.0 Safety

Before performing the method, the analyst must have read and be familiar with the CFIA Laboratory Safety Manual and Material Safety Data Sheets for the chemicals used.

13.0 Revision History

Version	Date	Description	Author
1	2012-09-06	New method	J. Soo

14.0 Bibliography

14.1 C. Sproll, W. Ruge, C. Andlauer, R. Godelmann and D. W. Lachenmeier (2008), HPLC analysis and safety assessment of coumarin in foods, *Food Chem,* 109, 2, 462-469.

1. Purpose and Scope

This method applies to the identification, confirmation and the quantitative determination of water-soluble colours in food by high performance liquid chromatography (HPLC). A list of permitted food colours in Canada, United States and in Europe, which can be analysed quantitatively, can be found in Table 4. The method has been validated for a wide range of preparation and food commodities, such as confectionary products, pastries and cookies, breakfast cereals, juices and drinks, chips, seasoning sauces, spices, fish, caviar, milk products and products which contain Japanese horseradish (wasabi).

2. Principle and Theory

Ion pair chromatography is performed by adding a counter ion to the mobile phase; thereby, forming a reversible complex with the water-soluble colours containing one or more functional groups, such as acidic or salt acidic moieties. The neutral complex thus formed is then separated by reverse-phase chromatography.

Detection is performed by scanning the wavelengths within the UV-Visible (UV-Vis) range, 190 nm to 950 nm. A UV-Visible spectrum is thus obtained for each colour. Subsequently, a chromatogram can be extracted at one of the acquisition wavelengths (408, 428, 506, 540 and 610 nm). Identification of the colours is achieved by overlay and comparison of the UV-Visible spectrum and from comparison of the retention times. Quantification is performed by external standard.

3. References

3.1 LCAQ-016: Determination of water-soluble food colours in foods by HPLC
3.2 J.F. Lawrence *et al*., Journal of Chromatography, 210 (1981), 168-173
3.3 F.E. Lancaster and J.F. Lawrence, Journal of Chromatography, 388 (1987) 248-252
3.4 F.E. Lancaster and J.F. Lawrence, Food Additives and Contaminants, 1999, Vol.16, No.9 381-390
3.5 S. Dixit *et al*., Journal of AOAC International Vol. 93, No.5 (2010), 1503-1514
3.6 M.C. Genarro *et al*., Journal of Chromatography A, 674 (1994), 281-299
3.7 NMKL, Nordic Committee on Food Analysis, No. 130 (1989)
3.8 A. Weisz *et al*., Journal of Chromatography A, 658 (1994) 505-510
3.9 B. Gandul-Rojas *et al*., Food Science and Technology (2011) – Accepted Manuscript
3.10 J.L. Garrido and M. Zapata, Chromatographia, Vol. 35, No.9-12, May/June 1993
3.11 J.L. Garrido and M. Zapata, Journal of High Resolution Chromatography, Vol.16 (1993), 229-233
3.12 J.D. Davis *et al*., Journal of Chromatography, 621 (1993), 105-109
3.13 A. Gratzfeld-Husgen *et al*., Agilent Technologies Application Note: Sensitive Analysis of Synthetic Colors using HPLC Diode-Array Detection at 190-950 nm
3.14 Health Canada's Food and Drugs Regulation, Part B, Division 6, sections B.06 and B.16, Table III and VIII
3.15 The Merck Index 11th Edition", Merck and Co. Inc., Rahway, USA, 1989.
3.16 The Sigma-Aldrich Handbook of Stains, Dyes and Indicators", Floyd J. Green, Sigma-Aldrich Corporation, USA, 1990.

4. Terminology and Definitions

HPLC: High Performance Liquid Chromatography

UV-Vis: Ultraviolet-Visible

DAD: Diode-Array Detector

CRM: Certified Reference Material

RM: Reference material

5. Material and Equipment

5.1 Three point decimal balance

5.2 Four point decimal balance

5.3 Beakers

5.4 Erlenmeyer flasks

5.5 Volumetric flasks

5.6 Graduated cylinders

5.7 Volumetric pipettes

5.8 Automatic pipettes

5.9 Dispensors

5.10 Mobile phase filtration system with 0.45 µm nylon membranes, or equivalent

5.11 Processor or mixer/blender (Polytron), or equivalent

5.12 Transfer pipettes

5.13 HPLC system (Agilent Technologies or equivalent equipped with a quaternary pump and an injector equipped with a needle wash option) with a diode array detector (DAD)

5.14 Chromatographic column : Poroshell SB-C18, 2.7 µm, 3.0 x 100 mm (Agilent Technologies), or equivalent

5.15 Guard column: SecurityGuard cartridge C18, 4 x 3.0 mm (Phenomenex), or equivalent

5.16 In-Line filters, 2 µm frits (Agilent Technologies), or equivalent

5.17 Ultrasonic bath

5.18 Magnetic bars

5.19 Magnetic stirring plate

5.20 Centrifuge

5.21 15 mL polypropylene tubes

5.22 HPLC vials and caps

5.23 0.45 µm nylon membrane

6. Reagents and solutions

NOTE: **Consult the respective MSDS sheet before using any of these products.**

6.1 Reagents

6.1.1. Methanol, HPLC grade or equivalent

6.1.2. Acetonitrile, HPLC grade or equivalent

6.1.3. Acetone, HPLC grade or equivalent

6.1.4. Ultra pure water, ASTM-I grade

6.1.5. Ammonium acetate, reagent grade or equivalent

6.1.6. Glacial acetic acid, reagent grade or equivalent

6.1.7. Alpha-amylase (from *Aspergillus oryzae*) > 60 units/mg solid (Sigma-Aldrich A9857 or equivalent)

6.1.8. Reference standards for water-soluble colours (See Table 11)

6.2 Solutions

6.2.1. Ammonium acetate solutions

6.2.1.1. 50 mM solution with 0.1% acetic acid :

Accurately weigh about 7.708 ± 0.100 g of ammonium acetate and transfer to a 2 L volumetric flask containing 500 mL of ultra pure water. Using a volumetric pipette, transfer 2.0 mL of glacial acetic acid. Mix well and complete to volume with ultra pure water.

6.2.1.2. 100 mM solution :

Accurately weigh about 15.416 ± 0.100 g of ammonium acetate and transfer to a 2 L volumetric flask containing 500 mL of ultra pure water. Mix well and complete to volume with ultra pure water.

6.2.1.3. 1 M solution :

Accurately weigh about 7.708 ± 0.100 g of ammonium acetate and transfer to a 100 mL volumetric flask containing 50 mL of ultra pure water. Mix well and complete to volume with ultra pure water. Filter the solution through a 0.45 µm nylon membrane.

6.2.2. 15 mg/mL alpha-amylase solution

Calculate the volume required for a batch. In a 1L beaker, accurately weigh the alpha-amylase and using a graduated cylinder add the ultra pure water to prepare a 15 mg/mL solution. Mix with the use of a magnetic stirring plate until the solution is homogenous.

NOTE: The water must be added slowly to minimise foaming.

6.2.3. Mobile phases

6.2.3.1. Mobile phase A:

Combine 970 mL of 50 mM acetate ammonium solution with 0.1% acetic acid (6.2.1.1) and 30 mL of methanol. Mix well and filter through a 0.45 µm nylon membrane.

6.2.3.2. Mobile phase B:

Combine 500 mL of 50 mM ammonium acetate solution with 0.1% acetic acid (6.2.1.1), 150 mL of methanol and 350 mL of acetonitrile. Mix well and filter through a 0.45 µm nylon membrane.

6.2.3.3. Mobile phase C:

Combine 50 mL of the filtered 1 M ammonium acetate solution (6.2.1.3), 250 mL of methanol, 200 mL of acetone. Mix using a magnetic stirring plate and cap the bottle using aluminum foil. Do not filter the solution (**CCP #1**). This solution is stable for 5 days, maximum.

7. Standards

NOTE: **The standard's purity must be considered when the concentration is calculated.**

7.1 Calibration curves: water-soluble colours

7.1.1. Stock solution for permitted food colours (200 µg/mL)

Accurately weigh about 10.00 mg of each permitted food colours (see table 6) in a 50 mL volumetric flask. Add 30 mL of ultra pure water and let sit in an ultrasonic bath for a minimum of 2 minutes. Complete to volume with ultra pure water. Wrap the flask with aluminium foil and keep refrigerated **(CCP #2)**. This stock solution is considered stable for a maximum of 70 days.

7.2 Working solutions: calibration curve

From the permitted colours stock solution (7.1.1), prepare a calibration curve at 5 concentration levels by diluting directly into the HPLC vials with ultra pure water (see Table 1). Vortex between each dilution. If the calibration curve solutions are not injected on the day of preparation, refrigerate and keep away from light **(CCP #3)**.

Table 1: Dilutions for calibration curve of permitted food colours

Solution	Conc. (µg/mL)	Volume (µL)	Volume of ultra pure water (µL)
5	50	250 of 7.1.1	750
4	5	100 of Sol. 5	900
3	0.5	100 of Sol. 4	900
2	0.05	100 of Sol. 3	900
1	0.025	250 of Sol. 2	250

Each concentration level must be injected twice. External standard quantification is applied and the calibration curves must be generated with the following parameters:

Type: Linear
Origin: Ignored
Weight: Quadratic (Amt); $1/X^2$

7.3 Creation of the UV-Vis spectral library

7.3.1. Reference solution

Use calibration curve solution #4 prepared in 7.2 (see Table 1) as the reference solution.

7.3.2 HPLC Analysis :

7.3.2.1 Spectral library

Inject the reference solution, for the creation and backup of the spectral library by following the method's chromatographic conditions.

NOTE: **The stock solution (see 7.1.1) can be injected according to the method's chromatographic conditions in order to assess the chromatographic purity of the standards. The chromatographic purity should be evaluated each time a new lot standard is used.**

7.3.2.2 Instrumental Qualification

Use and inject solution #4 (7.2 calibration curve) as the instrumental qualification solution. The injection also serves as retention time standard for permitted food colours.

7.3.3 Data analysis:

From the injection (well-defined peak and UV-Vis spectrum), save the UV-Vis spectrum in the library by identifying the standard's name, C.I. (Colour Index), retention time, wavelength of analysis, and the concentration of the reference solution.

8. Procedure

8.1 Quantitative analysis

NOTE: Check each sample to see if its ingredient list contains one of the following ingredients: starches (corn, tapioca, rice, etc.), modified starch or potatoes. If it does or if this information is not provided, proceed to step 8.1.2 following the weighing of the samples, if it doesn't, proceed to step 8.1.3. (CCP #4)

8.1.1. Weighings :

8.1.1.1. Accurately weigh approximately 10.000 g of each sample (5.000 g in the case of fish, caviar and spices) into a 125 mL erlenmyer flask. The samples must be weighed twice since they will be extracted using two different extraction solutions.

8.1.1.2. Add a magnetic bar to each erlenmeyer flask.

8.1.2. Samples which require enzymatic digestion (i.e. alpha-amylase)

8.1.2.1. Add 5 mL of 15 mg/mL alpha-amylase solution (6.2.2) to each sample.

8.1.2.2. To each sample, add 30 mL of 100 mM ammonium acetate solution (6.2.1.2). Use a magnetic stirring plate to mix each sample for a minimum of 15 minutes. Proceed to step 8.1.4

8.1.3. Samples for which enzymatic digestion is not required

8.1.3.1. Add to each sample, 40 mL of 100 mM ammonium acetate solution (6.2.1.2). Use a magnetic stirring plate to mix each sample for a minimum of 15 minutes. Proceed to step 8.1.4.

8.1.4. Extraction:

8.1.4.1. Extraction solution #1

Add 25 mL of 100 mM ammonium acetate solution (6.2.1.2) and 5 mL of methanol (extraction solution 1) to the first erlenmeyer flask. Let mix for a minimum of 15 minutes.

8.1.4.2. Extraction solution # 2

To the second erlenmneyer flask, add 30 mL of methanol. Let mix for a minimum of 15 minutes.

Table 2: Extraction summary

Step 1 (section 8.1.2 or 8.1.3)			**Step 2** (section 8.1.4)		
Enzymatic digestion	α-amylase (6.2.2)	100 mM ammonium acetate solution (6.2.1.2)	Extraction solution	100 mM ammonium acetate solution (6.2.1.2)	Methanol
α-amylase (8.1.2)	5 mL	30 mL	1	25 mL	5 mL
	5 mL	30 mL	2	---	30 mL
None (8.1.3)	---	40 mL	1	25 mL	5 mL
	---	40 mL	2	---	30 mL

8.1.5. Drain the remaining water in the ultrasonic bath and replace it with hot tap water (approximately 60 ± 10°C). Sonicate for a minimum of 15 minutes.

8.1.6. Transfer the solutions into individual 100 mL volumetric flasks. Rinse each erlenmeyer flask with two portions of approximately 10 mL of 100 mM ammonium acetate (6.2.1.2). Let cool until room temperature is reached. Complete each volumetric flask with 100 mM ammonium acetate solution (6.2.1.2). Mix well.

8.1.7. Transfer approximately 12 mL of each solution (8.1.6) to a 15 mL polypropylene tube. Centrifuge, at 22000 rcf (approx. 14000 rpm), for a minimum of 5 minutes at room temperature.

8.1.8. With the use of a transfer pipette, recover the supernatant. Avoid taking the floating fat layer at the surface of the liquid (visible on samples containing 2% fat or more). Transfer to a HPLC vial and inject on the chromatograph system.

9. Chromatography

9.1 Chromatographic conditions:

9.1.1. Mobile phase A: see 6.2.3.1

9.1.2. Mobile phase B: see 6.2.3.2

9.1.3. Mobile phase C: see 6.2.3.3

9.1.4. Column temperature: 60°C

9.1.5. Flow rate: 0.75 mL/min

9.1.6. Injection volume: 25 µL

9.1.7. Temperature of injector: 25°C

9.1.8. Needle wash: *Needle Port 5 sec.* Methanol/Water (50/50 v/v)

9.1.9. Gradient: Table 3

Table 3: Gradient

Time (min)	Phase A (%)	Phase B (%)	Phase C (%)
0	100	0	0
1	100	0	0
1.01	97	3	0
2	97	3	0
4	67	33	0
5	50	50	0
6	25	75	0
7	0	75	25
8	0	75	25
8.01	0	50	50
9	0	25	75
11	0	0	100
11.01	100	0	0
15	100	0	0

9.1.10. Detector: - Wavelength : 408, 428, 506, 540 et 610 nm
- Bandwidth: 16 nm
- Reference : none (off)

9.1.11. Post-Time : 1 min

Table 4: Specific wavelength for each quantifiable water-soluble colour

Food Colourants	CAS #	Color Index (C.I.)	Analysis λ (nm)
Tartrazine	1934-21-0	19140	428
Amaranth	915-67-3	16185	506
Indigo Carmine	860-22-0	73015	610
Sunset Yellow FCF	2783-94-0	15985	506
Allura Red	25956-17-6	16035	506
Ponceau SX	4548-53-2	14700	506
Fast Green FCF	2353-45-9	42053	610
Bleu Brillant FCF	3844-45-9	42090	610
Erythrosin B	15905-32-5	45430	540
Chlorophyllin	11006-34-1	75815	408
Ponceau 4R	2611-82-7	16255	506
Azorubine	3567-69-9	14720	506
Green S	3087-16-9	44090	610
Quinoline Yellow	8004-92-0	47005	408
Rhodamine B	81-88-9	45170	506
Patent Blue Violet	3536-49-0	42051	610

9.2 HPLC System

Equilibrate the HPLC system with mobile phase A for about 30 minutes prior to the start of the analysis. The reference solution (7.3.2) is injected prior to each analysis, serving as “instrumental qualification”.

NOTE: When a new column is installed on the HPLC system, let the mobile phase B (6.2.3.2) condition the column for 30 minutes before switching to mobile phase A (6.3.2.1) to equilibrate the system prior to the analysis.

9.3 Data analysis

9.3.1. Spectral library search

Extract the UV-Vis spectrum and conduct a library search of the integrated chromatographic peaks. This search (be it manual or automatic) should identify any colours with a minimum match factor of 900.

NOTE: **During the chromatograms reprocessing, Tartrazine, Amaranth and Indigo Carmine peaks are only analyzed when extraction solution #1 is used (8.1.4.1). When extraction solution #2 (8.1.4.2) is used, all other permitted dyes peaks are analyzed.**

9.3.2. Quantification (applies to compounds from Table 4 only)

Use the appropriate reprocessing (quantification) method according to the permitted water-soluble colours detected (**CCP #5**).

10. Results and Calculations

10.1 Samples

10.1.1. Record the amount obtained in µg/mL (W) in the working sheet.

10.1.2. Report the results as µg/g.

$$X\ (\mu g/g) = \frac{W\ (\mu g/mL) \times \text{Dilution Factor (mL)}}{\text{Weight of sample (g)}}$$

11. Quality Control

11.1 Certified or internal reference materials (CRM ou RM)

If throughout the series of analysis, samples are extracted according to 8.1.2 (with enzymatic digestion) and according to 8.1.3 (without enzymatic digestion), each corresponding control will be treated and extracted with both extraction solutions (8.1.4.1 and 8.1.4.2).

11.2 Reagent blank

11.2.1. Add 3 mL of methanol to a 15 mL polypropylene tube.

11.2.2. If the sample was enzymatically digested (8.1.2), add 1 mL of alpha-amylase solution (6.2.2)

11.2.3. Add the required volume (6 or 7 mL) of 100 mM ammonium acetate extraction solution. (6.2.1.2) to reach a final volume of 10 mL.

11.3 Inject HPLC vials filled with ultra pure water at a regular interval in the analysis run in order to minimise carry over.

11.4 Quality Control

Table 5 : Quality Control criteria

Control steps	Quality Control Criteria	Corrective action is the criteria is not met
Instrumental qualification	All peaks associated to permitted colours should be found within the spectral library	Manually identify peaks present in the chromatogram of the instrumental qualification injection
Correlation coefficients (R)	R ≥ 0.97 (but R ≥ 0.85 for Chlorophyllin)	If need be, repeat calibration curve injections for one or more points on the curve.
Reagent blank	≤ Quantification limit	1: Subtract peak areas obtained in the blank from samples in which reagents were used or 2: Repeat the analysis.

12. Critical Control Point (CCP)

Table 6 : Critical control points

No.	Section	Step	CCP
# 1	6	6.2.3.3	Mix mobile phase C using a magnetic stirring plate; do not mix by manual inversion. The solution is stable for a maximum of 5 days. Ensure the solution is wrapped with aluminium foil during storage.
# 2	7	7.1.1	The permitted food colours stock solution must be covered with aluminium foil and stored in the fridge. The solution is stable for 70 days.
# 3	7	7.2	If the calibration curve solutions are not injected on the day of preparation, they must be stored in a fridge and away from any source of light.
# 4	8	8.1	Enzymatic digestion must be performed for all samples containing one of the ingredients mentioned at section 8.1 or if the information is not available
# 5	9	9.3.2	Retention times and UV-Vis spectra are obtained using identical chromatographic parameters.

END OF DOCUMENT

1. Object and scope of application

This method is applicable to the determination of fat-soluble dyes by High-Pressure Liquid Chromatography (HPLC) in food commodities including the Sudan-type category of illegal dyes. The food commodities for which the method has been validated include sauces, (Tabasco sauce, Asian spicy sauces, etc.), food pastes and oil-based products (chilli paste, curry paste, etc.) powdered spices (chilli, paprika, turmeric and others) as well as salted eggs and oils. Further more, the method has been validated for Citrus Red 2 in fresh oranges, marmalades and orange juices.

2. Principle and theory

The fat-soluble dyes are extracted from the food samples by three (3) successive liquid-liquid extractions using tetrahydrofuran (THF). Following a manual Vortex mixing, sonication, Vortex mixing by plates, centrifugation and filtration, the liquid extract is concentrated by evaporation under a stream of nitrogen, re-dissolved in a minimum of THF, filtered and analyzed by HPLC with a diode-array detector in the UV-Visible (400 - 700 nm). Quantification is done by external standard calibration curve.

3. References

3.1 MET-027, Méthode de dosage des colorants liposolubles, ACIA-CFIA.

3.2 Lincolne Sutton and Wood Norwich Laboratory, Collaborative Trial 145 of a Method for the Detection and Determination of Sudan I in Chilli Products by HPLC, Method 145A, (2003).

3.3 H.-W. Sun *et al.*, J. Chromatogr. A, 1164 (2007) 120.

3.4 R. Noguerol-Cal *et al.*, J. Chromatogr. A 1179 (2008) 152.

3.5 Y. Uematsu *et al.*, Journal of AOAC International Vol. 90, No. 2, (2007) 437.

3.6 Application Note: Azo-dyes in Spices, Applied BioSystems, MDS Sciex, 2007.

3.7 Application Note: Determination of Sudan Dyes in Food Products by HPLC, Shimadzu Scientific Instruments, February 2006.

3.8 Application Note: A Rapid and Sensitive Analysis Method for Sudan Reds in Curry and Chili Powder using LC/MS/MS, Agilent Technologies, 2008.

4 Terminology et definitions

4.1 HPLC: High-Performance Liquid Chromatography

4.2 CRM: Certified reference material

4.3 RM : Reference material

5. Equipment and materials

5.1. Glassware and apparatus:

5.1.1. Filtration system for mobile phases: nylon membranes of 0.45 µm, or equivalent

5.1.2. Plastic syringes 1 mL or equivalent

5.1.3. Polytetrafluoroethylene (PTFE) membrane filters, 0.45 µm or equivalent

5.1.4. HPLC injection vials

5.1.5. Volumetric pipettes and automatic pipettes

5.1.6. Graduated cylinders of various volumes

5.1.7. 50 mL volumetric flasks (**CCP#1**)

5.1.8. Glass graduated 15 mL test tubes (**CCP#1**)

5.1.9. 50 mL plastic centrifugation tubes

5.1.10. Glass funnels (**CCP#1**)

5.1.11. Glass wool

5.1.12. Transfer pipettes

5.1.13. Dispenser

5.2. Auxilliary equipment:

5.2.1. Centrifuge

5.2.2. Polytron homogenizer, or equivalent.

5.2.3. Vortex mixer (manual and with plates)

5.2.4. Apparatus for evaporation under stream of nitrogen (e.g. *N-Evap*, or equivalent). (capable of maintaining a temperature of about 55°C)

5.2.5. Refrigerator

5.2.6. Freezer

5.2.7. Ultrasonic bath

5.3. Analytical instruments:

5.3.1 HPLC system (Agilent Technologies or equivalent) with diode-array detector (DAD)

5.3.2 Reverse-phase C-18 HPLC column.
(Agilent Technologies, Zorbax SB-C18, *Rapid Resolution HT* 4,6 x 50 mm, 1.8 µm. or equivalent).

5.3.3 Reverse-phase C-18 column guard.
(Phenomenex Security-Guard cartridge C18, 4 x 3.0 mm, or equivalent).

6. Reagents and solutions

NOTE on security: Read the MSDS of the products prior to use.

6.1. Reagents :

6.1.1 Methanol (MeOH), HPLC grade (or equivalent)

6.1.2 Acetonitrile (ACN), HPLC grade (or equivalent)

6.1.3 Tetrahydrofuran (THF), HPLC grade, ACS grade (or equivalent)

6.1.4 Trifluoroacetic acid (TFA), reactive grade or higher; ≥98% (or equivalent)

6.1.5 Ultrapure water, ASTM-I grade

6.1.6 Dodecyltrimethylammonium bromide (DDTMABr), reagent grade (or equivalent)

6.1.7 Tetrabutylammonium phosphate monobasic (TBAHP), 1.0 M solution in water, reagent grade (or equivalent)

6.1.8 Sodium chloride (NaCl), ACS grade (or equivalent)

6.1.9 Ethanol 95% (EtOH 95%), reagent grade (or equivalent)

6.2. Solutions:

6.2.1 Mobile phases:

6.2.1.1 Mobile phase A:

Into a 1000 mL volumetric flask, weigh accurately about 3.08 g of DDTMABr and dissolve with 800 mL Ultra pure water. Into the same flask, add 60 mL of 1.0 M TBAHP solution with a graduated cylinder. Add 1.5 mL of TFA and mix. Let cool the solution to room temperature and fill up to volume with Ultra pure water. Filter through a nylon membrane filter, 0.45 µm (**CCP#2**)

6.2.1.2 Mobile phase B:

Into a 1000 mL volumetric flask half-filled with ACN, add 1.5 mL of TFA and mix. Fill up to volume with ACN. (**CCP#2**)

6.2.1.3 Mobile phase C:

Into a 2000 mL volumetric flask half-filled with MeOH, add 3.0 mL of TFA and mix. Fill up to volume with MeOH. (**CCP#2**)

NOTE: Mobile phases have to be used within 7 days of preparation.

7. Standards:

7.1. Standards:

Table 1: List of quantified fat-soluble dyes

Fat-Soluble Dye	# CAS	Color Index (C.I.)	Analysis λ (nm)
Sudan I	842-07-9	12055	473
Sudan II	3118-97-6	12140	525
Sudan III	85-86-9	26100	525
Sudan IV	85-83-6	26105	525
Sudan Red B	3176-79-2	26110	525
Sudan Red 7B	6368-72-5	26050	525
Sudan Red G	1229-55-6	12150	525
Sudan Orange G	2051-85-6	11920	400
Sudan Blue II	17354-14-2	61554	625
Solvent Blue 59	6994-46-3	61552	625
Toluidine Red	2425-85-6	12120	525
Para Red	6410-10-2	12070	473
Methyl Yellow	60-11-7	11020	400
Metanil Yellow [1]	587-98-4	13065	400
Sudan Black B	4197-25-5	26150	625
Citrus Red 2	6358-53-8	12156	525

[1]: Water-Soluble dye

NOTE: The following dyes: Citrus Red 2, Sudan Black B and Metanil Yellow will be prepared if required. The corresponding calibration curves will be prepared if required.

7.2. 50 µg/mL standard stock solution:

7.2.1 Standard stock solution of the first 13 dyes (mix solution)

According to the respective purity of each standard, and using a different weighing dishes, accurately weigh about 10 mg of each of the thirteen (13) first dyes (see Table 1). Transfer into a 200 mL volumetric flask. Add about 100 mL of THF and mix slightly to dissolve all the dyes. Sonicate the solution for a few minutes, fill up to volume with THF and use a glass stopper. Wrap the flask with aluminium foil to avoid light and store in the refrigerator (**CCP#3**).

7.2.2 Standard stock solution of Citrus Red 2

According to the respective standard purity, accurately weigh about 10 mg of Citrus Red 2 into a weighing dish. Transfer into a 200 mL volumetric flask. Then, add about 100 mL of THF and mix slightly to dissolve the dye. Sonicate the solution for a few minutes, fill up to volume with THF and use a glass stopper. Wrap the flask with aluminium foil to avoid light and store in the refrigerator (**CCP#3**).

7.2.3 Standard stock solution of Sudan Black B

According to the respective standard purity, accurately weigh about 10 mg of Sudan Black B into a weighing dish. Transfer into a 200 mL volumetric flask. Then, add about 100 mL of THF and mix slightly to dissolve the dye. Sonicate the solution for a few minutes, fill up to volume with THF and use a glass stopper. Wrap the flask with aluminium foil to avoid light and store in the refrigerator (**CCP#3**).

7.2.4 Standard stock solution of Metanil Yellow

According to the respective purity of Metanil Yellow, accurately weigh about 10 mg of Metanil Yellow standard into a weighing dish. Transfer into a 200 mL volumetric flask and rinse with a maximum of 10-15 mL of ultra pure water. Mix gently in order to wet and dissolve the dye. Then, add about 100 mL of THF. Sonicate the solution for a few minutes, fill up to volume with THF and use a glass stopper. Wrap the flask with aluminium foil to avoid light and store in the refrigerator (**CCP#3**).

NOTE: **Standard stock solutions: mix solution (7.2.1), Citrus Red 2 (7.2.2), Sudan Black B (7.2.3), and Metanil Yellow (7.2.4) are stable when kept in the refrigerator and can be used for a period up to 3 months.**

7.3. Calibration curve: working solutions (solution mix)

Into individual 10 mL volumetric flask, dilute each working solution to the desired concentrations. Fill up to volume with THF, filter through a PTFE membrane filter, 0.45 µm, and fill the HPLC vial. Working solutions are injected with analysis conditions and can be used until stock solutions are stable.

7.4. Calibration curve: working solutions

Table 2: Working solutions for calibration curves

Working Solution	Pipetted Volume (mL)	Final Volume (mL)	Solution	Final Concentration (µg/mL)
7[2]	—	—	solutions 7.2.1, (and/or if necessary 7.2.2., 7.2.3 and 7.2.4)	50
6[2]	5.0	10	solutions 7.2.1, (and/or if necessary 7.2.2., 7.2.3 and 7.2.4)	25
5	2.0	10	solutions 7.2.1, (and/or if necessary 7.2.2., 7.2.3 and 7.2.4)	10
4	0.5	10	solutions 7.2.1, (and/or if necessary 7.2.2., 7.2.3 and 7.2.4)	2.5
3	2.0	10	solution 7.4 # 4	0.5
2	0.5	10	solution 7.4 # 4	0.125
1	2.0	10	solution 7.4 # 2	0.025

[2]: Working solutions prepared individually, if 7.2.3 and/or 7.2.4 are necessary

7.5. Inject all working solutions for the calibration curves.

7.6. Following the reprocessing of all calibration curves chromatograms, the curve must be generated with the following parameters:

Type: Linear
Origin: Ignore
Weight: Quadratic (Amnt)

7.7. HPLC Performance Verification solution:

7.7.1 Instrumental Qualification (IQ)

Inject solutions #5 (prepared as per 7.2.1 and/or if necessary as per 7.2.2, .7.2.3 and 7.2.4; see Table 2). These solutions are injected as a verification performance tool and also as retention time standard in each analysis run.

8. Procedure

8.1. Sample preparation:

8.1.1. Manually mix portions of dry or powdered samples in order to prevent the production of potentially irritating aerosols (e.g. dried spices, powders, etc.).

8.1.2. Gently heat the samples of oils (e.g. palm oil) in order to ensure homogeneity prior to perform the sampling.

8.1.3. Homogenize all other samples with a food processor or other appropriate mixer.

8.1.4. Keep the prepared samples in sealed containers at room temperature, in a refrigerator or in a freezer, with respect to the samples.

8.1.5. Preparation for citrus :

8.1.5.1. Tare weight container on an analytical balance.

8.1.5.2. In a tared container put a minimum of 6 for big citrus fruits and 12 for smaller.

8.1.5.3. Determine the citrus fruit average weight using the citrus fruits total weight.

8.1.5.4. Peel the citrus fruits.

8.1.5.5. Weigh all peel of citrus fruits.

8.1.5.6. Determine the peel average weight using the peel total weight.

8.1.5.7. Homogenize the peel samples with a food processor.

8.1.5.8. Keep samples in freezer until analysis

8.2. Extraction procedure:

8.2.1. With respect to the nature of the sample to analyze, accurately weigh about 5 g of sample homogenate into a 50 mL plastic centrifugation tube. Analyze each sample in duplicate.

NOTE: If it's impossible to weigh 5 g of sample, you need to adjust the final dilution volume.

8.2.2. Add one spatula (approximately 1 or 2 g) of NaCl.

8.2.3. Add 15 mL of the extraction solvent (THF) to wet the sample and mix with Vortex for a few seconds.

8.2.4. Place all the 50 mL centrifugation tubes on a test tubes rack into the sonic bath and sonicate for approximately 15 minutes.

8.2.5. Then, using the Vortex mixer with plates, proceed for the mixing for approximately 15 more minutes (**CCP#4**).

8.2.6. Centrifuge the samples at 10000 rpm for approximately 5 minutes. Filter the supernatant liquid in a glass funnel with a glass wool plug into a 50 mL volumetric flask. If necessary, use pasteur pipettes to transfer the liquid phase.

8.2.7. Repeat steps 8.2.4 to 8.2.7.

8.2.8. For the last extraction, add only 10 mL of THF and repeat steps 8.2.5 to 8.2.7.

8.2.9. Fill up to volume the 50 mL volumetric flask with THF.

8.2.10. If samples are liquid or solid oil (oil, shortening, etc.), the 50 mL volumetric flask (8.2.9) should be store in a freezer overnight. If not, continue to step 8.2.14.

8.2.11 Transfer approximately 20 mL of cold extract into a 50 mL plastic centrifugation tube. Carefully transfer less precipitate as possible. Transfer should be done only once (**PCC#5**).

8.2.12 Cool to room temperature and follow 8.2.14.

8.2.13 Transfer 15 mL of the extract solution (8.2.9 or 8.2.12) into a 15 mL glass test tube using the 15 mL graduation. Using the N-Evap apparatus, evaporate the THF under a stream of nitrogen until the 1 mL mark. Do not evaporate to dryness. During the evaporation process, adjust the needle's height to ensure that the proper height is reached without any loss by extract splash (**CCP#6**).

NOTE: Verify *N-Evap* temperature using a calibrated thermometer.

8.2.14 Re-dissolve the concentrated extract, and all deposits if present, by adding THF at the 3 mL mark or at the final dilution volume according to the weigh (8.2.1). If necessary, use the sonic bath to help. Mix the extract using the manual Vortex mixer for a few seconds (**CCP#7**).

8.2.15 Filter the extract through a PTFE membrane filter, 0.45 µm and put in HPLC vial.

8.3 Creation of the spectral library

8.3.1 Reference solutions:

Use the calibration curves working solutions #5 prepared in 7.4 (see Table 2) as reference solutions.

8.3.2 HPLC Analysis:

8.3.2.1 Spectral Library

Inject the reference solutions (8.3.1) to create the spectral library by following the operating conditions of the method.

NOTE: The stock solutions (7.2.1 to 7.2.4) could also be injected using the operating conditions of the method in order to establish the standard's purity. The chromatographic purity has to be evaluated whenever a new lot of standard is used.

8.3.2.2 Instrumental Qualification (IQ)

Use and inject the solutions #5 (7.4; Table 2 – calibration curves) as IQ solutions for performance verification. These injections are also used as retention time standards.

8.3.3 Chromatograms reprocessing:

From each injections (dye peaks and well-defined UV-Visible spectra), backup all spectra of the dye standards of interest along with the Color Index No., the retention time, the wavelength of analysis and the concentration of the reference solution.

8.3.3.1 Any positive result has to be verified and confirmed by overlay and comparison of UV-Visible spectra using the spectral library. Manually-made library search is performed with respective spectral range adjustment.

8.3.4 Quantification of the fat-soluble dyes is performed using an external standard calibration curve and with the concentration in µg/mL.

9. Chromatographic system

9.1. Operating Conditions:

9.1.1. Mobile phases:

Mobile phase A: 10 mM dodecyltrimethylammonium bromide (DDTMABr), 60 mM tetrabutylammonium hydrogen phosphate (TBAHP) and 0.15% trifluoroacetic acid (TFA) aqueous solution
Mobile phase B: 0.15% TFA in acetonitrile
Mobile phase C: 0.15% TFA in methanol

9.1.2. Gradient:

Table 3: Gradient used

t (min)	Mobile phase A (%)	Mobile phase B (%)	Mobile phase C (%)
0.00	60	15	25
2.00	60	15	25
3.00	40	25	35
4.00	35	30	35
6.00	30	30	40
7.00	20	30	50
8.00	20	10	70
11.00	20	10	70
11.01	0	0	100
14.00	0	0	100

9.1.3. Flow rate: 1.5 mL/min

9.1.4. Stop time: 14 min

9.1.5. Post-time: 3 min

9.1.6. Injection volume: 5 µL (with needle rinse)

9.1.7. Needle rinse: *Needle Port 5 sec* (EtOH 95%)

9.1.8. Injection compartment temperature: approximately 25°C or ambient temperature

9.1.9. Column heater temperature: 50°C

9.1.10. Wavelengths of interest: the detector signal is monitored at 400, 473, 525 and 625 nm with 16-nm bandwidth and reference at OFF. Spectra are recorded between 200 and 800 nm with 2-nm intervals.

9.2. Equilibrate HPLC system as described in section 9.1.

9.3. Check for instrumental performance by injection of solution 7.7.1

9.4. Inject the performance verification solution (IQ), the solvent blank, and the sample extracts.

10. Calculations

10.1. Results for sample analysis:

10.1.1. Report the results in µg/mL.

10.1.2. Report the final results in µg/g.

$$Concentration\ (\mu g/g) = \frac{HPLC\ Concentration\ (\mu g/mL) \times Dilution\ Volume\ (mL)}{Sample\ Weight\ (g)}$$

Where: *Dilution Volume* =

$$\frac{Initial\ Volume\ (50\ mL) \times Final\ Dilution\ Volume\ (3\ mL)}{Transfered\ Volume\ (15\ mL)}$$

10.1.3. For citrus fruit, report the final results in µg/g citrus fruit.

$$Concentration(\mu g/g\ Citrus\ Fruit) = Concentration(\mu g/g\ of\ Peel) \times \frac{Peel\ Average\ Weight(g)}{Citrus\ Fruit\ Average\ Weight(g)}$$

11. Quality Control

11.1. For each series of analyses, one of the the performance verification solution (7.7.1) is injected to adjust retention times. All fat-soluble dye peaks should be detected and identified from the calibration table. It could be useful to check peaks using the library, if necessary.

11.2. A solvent blank (THF) is analyzed with each set of samples analyses.

11.3. One certified reference material (CRM) or reference material (RM) is used in each batch.

11.4. Quality Control Criteria

Table 4: Quality Control Criteria

Control steps	Quality Control criteria	Corrective action if criterion is not met
Instrumental Qualification (IQ)	All fat-soluble dye peaks included in the IQ mixture should be detected and identified.	The IQ mixture must be prepared again (7.7.1), system must be verified
Correlation Coefficient R	R ≥ 0.98	Repeat of one or all the calibration curve points, if necessary
Solvent Blank	≤ Quantification Limit (LOQ)	Subtract the area obtained from the solvent blank in each sample.
Duplicates	≤ 20% between results [(highest - lowest) / lowest] for positive results only	Repeat the sample in duplicate.

13. Critical Control Points (CCP)

Table 5: CCP

No.	Section	Paragraph	Critical Control Points (CCP)
1	5	5.1	Glassware should be rinsed with ethanol after use.
2	6	6.2.1.1 to 6.2.1.3	Mobile phases have to be used within 7 days of preparation because of the presence of TFA.
3	7	7.2.1 to 7.2.4	About 2 or 3 minutes sonication time might be necessary to ensure that all dyes in the stock solution are completely dissolved.
4	8	8.2.6	Ensure that the centrifugation tubes are tightly stoppered when they are placed between the plates of the Vortex mixer. Don't put too much pressure when you place the test between the plates.
5	8	8.2.11	Only one extract transfer should be done.
6	8	8.2.13	During the evaporation process, adjust the needle's height to ensure that the proper height is reached without any loss by extract splash.
7	8	8.2.14	Re-dissolve the concentrated extract, and all deposits if present, by adding THF until the 3-mL mark on the graduated test tube. Use the sonication bath if necessary.

End of Document

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 Polytron Homogenizer

5.1.5 50 mL Polypropylene Tubes

5.1.6 Balance capable of ±0.01 gram accuracy

5.1.7 Wrist action rotator

5.1.8 Centrifuge capable of 4000 g.

5.1.9 Funnels

5.1.10 Glass wool

5.1.11 Scintillation Vials

5.1.12 Sep Pak Plus SPE cartridges

5.1.13 Vacuum Manifold

5.1.14 6 mL SPE reservoirs

5.1.15 15 mL disposable tubes

5.1.16 15 mL graduated centrifuge tubes with stoppers

5.1.17 Vortex mixer

5.1.18 Nylon syringe filters, 0.2 µm pore size or equivalent

5.1.19 Ultra Performance Liquid Chromatography System

5.1.19.1 LC Pump System capable of providing flow rates up to 3.0 mL/min and at pressures of at least 3000 psi.

5.1.19.2 Autosampling system able to communicate with the pump and data system and providing injection volumes up to 100 µl.

5.1.19.3 Column oven capable of maintaining column temperatures of 60°C

5.1.19.4 LC Column: Waters BEH C18 or equivalent

5.1.19.5 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 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 and add 5.0 g $NaHSO_3$

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

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 and bring up to volume with DIW

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 and bring up to volume with DIW

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).

5.2.12 LC Mobile Phase: 60% MeCN with 0.01 M Potassium phosphate buffer. Mix 100 mL of phosphate buffer solution with 600 mL MeCN and make final volume 1.0 L with DIW.

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

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 MSDS's 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.

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 from potato sprouts supplied from Sigma - Aldrich and bring up to final volume of 25 mL in a volumetric flask with 0.1M KH_2PO_4.

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

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.

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.

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 Robot Coupe food processor, until homogenous. Transfer homogenate to a plastic container with a lid and store at ≤-18°C.

8.3 Extraction

8.3.1 Completely thaw samples and thoroughly mix the sample with a polytron homogenizer

8.3.2 Immediately weigh 5 g accurately into a 50mL 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 (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 extract solution to a glass 15 mL tube

8.4.3 Load 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 UPLC-PDA Conditions

8.5.1 Operate UPLC-PDA 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: Waters® UPLC BEH C18 1.7 µm, 2.1 x 50 mm
Column Temperature: 60ºC
Mobile Phase: 60% MeCN with 0.01 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 (µg/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.

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

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.

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

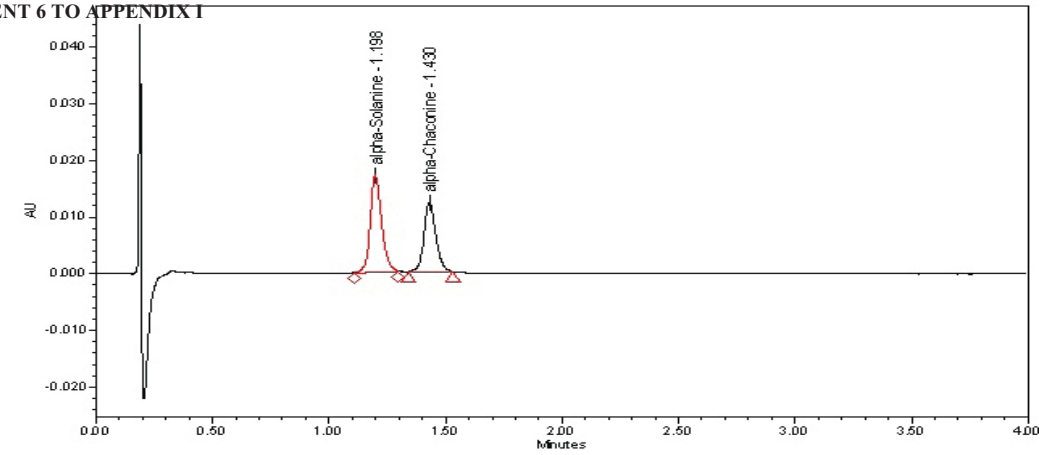

Figure 1: Chromatogram of 10 µg/mL Standard showing expected retention time and peak shape based on the conditions set in section 8.4

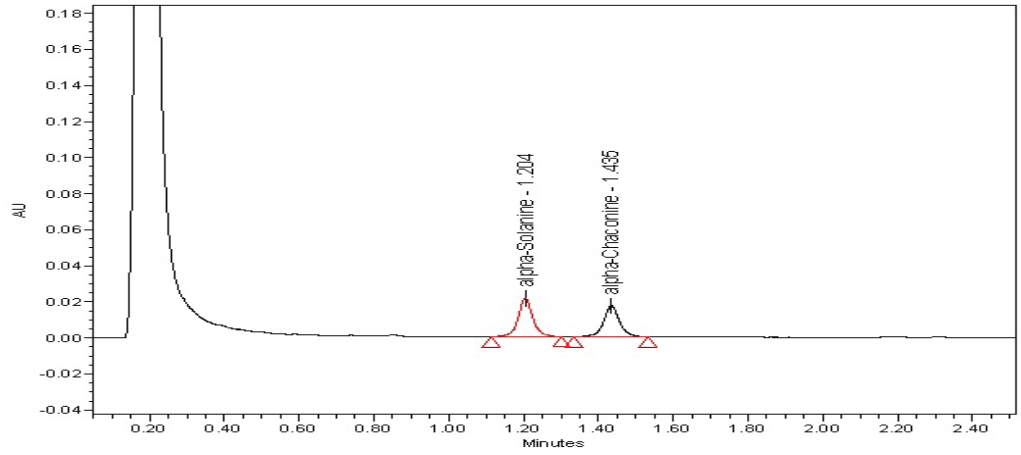

Figure 2: Example of a high level naturally incurred sample (15.2 mg/100g α-Solanine and 17.9 mg/100g α-Chaconine; Total = 33 mg/100g)

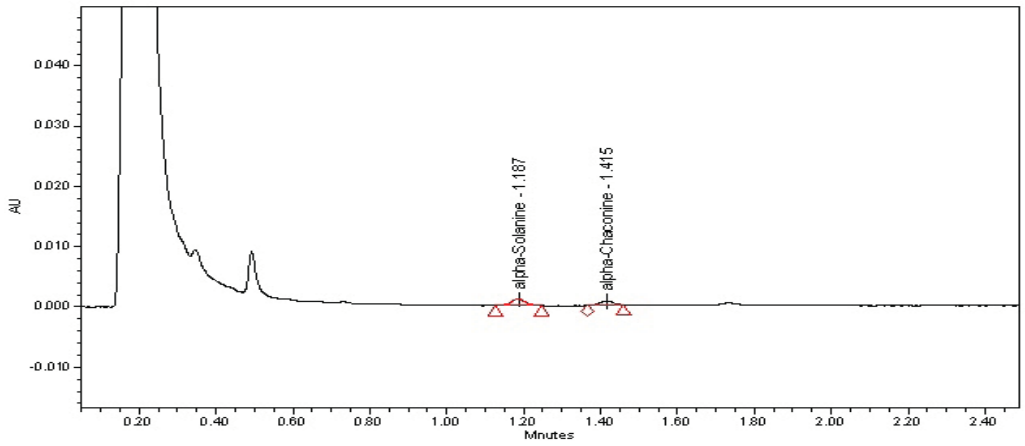

Figure 3: Example of a low level sample (0.7 mg/100g α-Solanine and 0.5 mg/100g α-Chaconine; Total = 1.2 mg/100g)

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Burnaby Laboratory Methods Manual

Meth Name: BFCL-047	Approved: _____ Science Manager Signature and Date
Document Control: _____ Unit Control #	Reference: Multiresidue mycotoxin analysis in wheat, barley, oats, rye and maize grain by high-performance liquid chromatography - tandem mass spectrometry, Martos et al., World Mycotoxin Journal, August 2010; 3 (3): 205-223

Multimycotoxin Analysis in Cereal Grains by HPLC-MS/MS

Scope and Application

This is a mycotoxin screen method intended to be used for survey work and is not appropriate for regulatory purposes. It is applicable to the simultaneous analysis of 24 mycotoxins in wheat flour, corn flour, oat flour, wheat bran and oat bran in the Burnaby Chemistry Lab. Included in this analysis are aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2), sterigmatocystin (STE), cyclopiazonic acid (CPA), ochratoxin A (OTA), deoxynivalenol (DON), nivalenol (NIV), fusarenone-X (FUS-X), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), neosolaniol (NEO), diacetoxyscirpenol (DAS), HT-2 toxin (HT-2), T-2 toxin (T-2), fumonisins B_1 (FB_1), B_2 (FB_2), B_3 (FB_3), zearalenone (ZEA), α-zearalenol (α-ZOL), β-zearalenol (β-ZOL), ergocristine, ergocryptine, and ergosine.

Principle

A known weight of solid sample is extracted with acetonitrile / water solution in a stomacher. The extract is centrifuged and the supernatant is diluted with water and filtered through a 0.2 µm PTFE filter. The filtered solution is analysed by high performance liquid chromatography with tandem mass spectrometric detection (HPLC-MS/MS).

Safety

Mycotoxins are secondary fungal metabolites that can cause disease in animals and humans. There are a total of 24 different mycotoxins that are included in this method. Potential dangers to human health exist with all of them. For example, OTA causes kidney and liver damage and is a probable carcinogen. DON causes damage to the gastrointestinal tract, lymphatic system, blood and immune system. Therefore, personal protective equipment such as a lab coat, gloves, and safety glasses should be worn at all times. Standard and sample preparation should be carried out in a fume hood.

The following documents must be read by the analyst prior to receiving training on the performance of this method:

- Job Hazard Analysis (JHA) Mycotoxins Analysis (RDIMS #2526451)

- JHA Sample Preparation (RDIMS #2526481)
- Safe Work Practice (SWP) 1 Handling and Working with Chemicals (RDIMS #1428175)
- SWP 2 Using Concentrated Acids (RDIMS #1428179)
- SWP 3 Using Solvents (RDIMS #1428180)
- MSDSs pertaining to each of the 24 mycotoxins in this method

Method Description

1.0 Equipment / Materials

1.1 Stomacher, Seward Stomacher 80 laboratory blender or equivalent

1.2 UV-VIS Spectrophotometer Agilent 8453 or equivalent

1.3 Mills or grinders

1.4 Eppendorf 5430R centrifuge or equivalent

1.5 Acrodisc CR syringe filters with 0.2 µm PTFE membrane or equivalent

1.6 Miscellaneous laboratory supplies

2.0 Reagents

2.1 Methanol (MeOH) and acetonitrile (ACN): HPLC grade or higher.

2.2 Extraction solution: acetonitrile / water (80 / 20)

2.3 Stock standard solution of OTA is prepared in toluene / acetic acid (99 / 1)

Stock standard solutions of all other mycotoxins are prepared in methanol or acetonitrile.

2.4 Diluents: Methanol / water (20 / 80)
Milli-Q water

2.5 Ascorbic Acid Solution 1 mg/mL

Example Preparation: dissolve 10 mg ascorbic acid in 10 mL Milli-Q water to produce a 1 mg/mL solution.

2.6 Ammonium Hydroxide (ammonia solution)

3.0 Standards

A mycotoxin standard that is either in powder form or dry film is dissolved in an appropriate solvent and its concentration is determined by UV-Vis Spectroscopy providing that maximum absorbance (λ_{max}) and molar extinction coefficient (ε) data is available. Examples of how this could be done are provided in sections 3.1, 3.2 and 3.3 below for the determination of DON,

OTA and DAS respectively. Please see Appendix B for maximum absorbance (λ_{max}) and molar extinction coefficient (ε) values for some of the mycotoxins.

For mycotoxins that are obtained from suppliers in solution form, use the concentration value that is found in the accompanying certificate of analysis.

After the concentrations of the individual mycotoxins are determined, two master mixes are prepared. These are called Master Mix 1 containing all of the mycotoxins except for ergot alkaloids and Master Mix 2 which is made of the ergot alkaloids. These two mixes are used for spiking of QC samples as well as for preparation of calibration curve standards. See sections 3.3.1 and 3.3.2 for examples of how the mixes may be prepared.

3.1 DON Standards

3.1.1 DON Stock Solution (approximately 500 µg/mL in ACN):

Dissolve the contents of 5 mg DON vial (Sigma product #D0156-5, or equivalent) in acetonitrile and transfer into a 10 mL volumetric flask. Fill up to mark with acetonitrile. Determine the exact concentration by UV absorbance as follows.

Prepare DON UV quantitation solution to quantitate the DON Stock Solution by UV absorbance. Transfer 100 µL of DON stock solution to a 2 mL volumetric flask. Make to volume with acetonitrile. Measure the UV absorbance of this solution against an acetonitrile solvent blank. Calculate the concentration of DON stock solution by maximum UV absorbance at wavelength close to 218 nm using the following equation:

$$C_{stock}\ \mu g/mL = A \times MW \times 1000 / 6400 / b \times (2 / 0.1) = A \times 925.9$$

Where A = maximum UV absorbance of the DON UV quantitation solution
MW = 296.3 g/mol
b = path length of the cuvette (cm)

3.1.2 DON Intermediate Standard (100 µg/mL DON in ACN)

Prepare 10 mL at a concentration of 100 µg/mL in ACN. To do this, calculate the exact volume to remove from the stock solution.

Volume of stock in mL to be diluted = (100 µg/mL x 10 mL) / C_{stock} µg/mL

Pipette the required volume of stock standard into a 10 mL volumetric flask and add ACN to the mark.

3.2 OTA Standard

3.2.1 OTA Stock Standard (ca. 40 µg/mL in toluene with 1% acetic acid)

Dissolve the contents of 1 mg OTA vial (Sigma product #O-1877) in toluene with 1% acetic acid and transfer into a 25 mL volumetric flask. Add solution to the mark. Determine the exact concentration by maximum UV absorbance at wavelength close to 333 nm using the following equation:

$$C_{stock}\ \mu g/mL = A \times MW \times 1000 / 5440 / b = A_{max} \times 74.23$$

Where A = maximum UV absorbance of the OTA stock standard
MW = 403.8 g/mol
b = path length of the cuvette (cm)

3.3 Diacetoxyscirpenol Standards

3.1.1 DAS Stock Standard (approximately 100 µg/mL in MeOH):

Dissolve the dry film (Micotox product #MDAS-1) in methanol to make up a final concentration of about 100 µg/mL DAS. Transfer the dissolved standard solution into an amber vial. Measure UV absorbance against a methanol solvent blank and calculate the actual concentration of DAS in this solution by maximum UV absorbance at wavelength close to 202 nm using the following equation:

$$C_{stock}\ \mu g/mL = A \times MW \times 1000 / 2487 / b = A_{max} \times 147.33$$

Where A = maximum UV absorbance of the DAS UV quantitation solution
MW = 366.4 g/mol
b = path length of the cuvette (cm)

3.3 Master Mixes

Sections 3.3.1 and 3.3.2 show an example procedure for preparation of two master mixes to be used for spiking of samples and for preparation of calibration standards. The exact volume of each mycotoxin stock standard required to produce its final concentration the Master Mix is determined as follows:

$$V_i = (C_f \times V_f) / C_i$$

V_i is the volume of mycotoxin stock standard to be added to the mycotoxin master mix
V_f is the final volume of mycotoxin master mix
C_i is the concentration of mycotoxin stock standard
C_f is the final concentration of mycotoxin in the mycotoxin master mix

3.3.1 Master Mix 1.

Standard Name	*Lot Number*	*Concentration µg/mL*	*Volume Used (µL)*	*Total Volume (µL)*	*Final Conc. (µg/mL)*
[a]Ochratoxin A	125K4063	55	29.1	4000	0.4
Aflatoxin B1	039K4047	13.75	116.4		0.4
Aflatoxin B2	079K4041	11.74	136.3		0.4
Aflatoxin G1	069K4012	15.61	102.5		0.4
Aflatoxin G2	050M4071	10.78	148.4		0.4
Sterigmatocystin	L10333B	50	32.0		0.4
Cyclopiazonic Acid	FT-CPA017	10.7	150.2		0.4
T-2 Toxin	FT-T006	80.8	198.0		4
HT-2 Toxin	FT-HT-003	98.1	163.1		4

Standard Name	*Lot Number*	*Concentration µg/mL*	*Volume Used (µL)*	*Total Volume (µL)*	*Final Conc. (µg/mL)*
Diacetoxyscirpenol	FT-DAS003	110.2	145.2		4
Neosolaniol	FT-NS001	145.7	109.8		4
Deoxynivalenol	097K4010	100	160.0		4
[b]3-Acetyldeoxynivalenol	L10301D	100.5	79.6		2
[b]15-Acetyldeoxynivalenol	7073X	107.4	74.5		2
Fusarenone X	L10301B	100.1	159.8		4
Zearalenone	FT-Z008	98.4	162.6		4
α-ZOL	031M4101V	100	160.0		4
β-ZOL	098K4129	100	160.0		4
Nivalenol	L10301G	100.6	159.0		4
Fumonisin B1	L10052A	50.8	315.0		4
Fumonisin B2	L10052B	50	320.0		4
Fumonisin B3	L10301H	50.4	317.5		4
Methanol Volume (µL)	68037	N/A	601.0		N/A

[a] Transfer the exact volume of Ochratoxin A (in toluene with 1% acetic acid) to a 4 mL amber vial and evaporate solvent under nitrogen at <37°C. Add methanol to replace the volume that was just evaporated. Continue adding the other standards to the mix followed by methanol to make up to 4 mL.

[b] 3-Acetyldeoxynivalenol and 15-Acetyldeoxynivalenol are quantified as a sum, therefore to obtain a total concentration of 4 µg/mL in the Master Mix, each AcDON should be made up at 2 µg/mL.

3.3.2 Master Mix 2 (Ergot Alkaloids).

Standard Name	*Lot Number*	*Concentration µg/mL*	*Volume Used (µL)*	*Total Volume (µL)*	*Final Concentration (µg/mL)*
Ergocristine	L10273E	100.4	159.4	4000	4
Ergocryptine	L10273C	101.8	157.2		4
Ergosine	L10273F	100.6	159.0		4
Acetonitrile	50343	N/A	3524.4		N/A

3.4 Suggested Procedure for Preparation of Calibration Standards

In Table A, Master Mix 1 and Master Mix 2 are combined to form a single multi-mycotoxin mixed standard solution. In Table B, this mix is serially diluted to form a seven-point calibration curve.

Table A. Multimycotoxin Mixed Standard Solution for Calibration Curve Preparation.

Standard Mix Components	*Final Concentration (ng/mL)*	*Volume of Master Mix 1 (µL)*	*Volume of Master Mix 2 (µL)*	*Volume MeOH (µL)*	*Total Vol (µL)*

Standard Mix Components	*Final Concentration (ng/mL)*	*Volume of Master Mix 1 (µL)*	*Volume of Master Mix 2 (µL)*	*Volume MeOH (µL)*	*Total Vol (µL)*
[†TCTs + FBs + Ergot Alkaloids] / [Afla + STE + OTA + CPA]	200 / 20	50	50	900	1000

†TCTs refers to trichothecenes, which include DON, NIV, FUS-X, ADONs, NEO, DAS, HT-2, and T-2.

Table B. Multimycotoxin Calibration Curve Preparation.

[TCTs + FBs + Ergots Alkaloids] / [Afla + STE + OTA + CPA] Concentration (ng/mL)	*Mixed Standard Used (ng/mL)*	*Volume Used (µL)*	*Volume Water (µL)*	*Volume 20% MeOH (µL)*	*Volume MeOH (µL)*	*Final Volume (µL)*
40 / 4	200 / 20	200	800	0	0	1000
20 / 2	200 / 20	100	800	0	100	1000
10 / 1	200 / 20	50	800	0	150	1000
5 / 0.5	40 / 4	125	0	875	0	1000
2.5 / 0.25	20 / 2	125	0	875	0	1000
1.25 / 0.125	10 / 1	125	0	875	0	1000
0.625 / 0.0625	5 / 0.5	125	0	875	0	1000

Procedure

4.0 Sample Preparation

4.1 Samples are usually submitted by CFIA inspectors, according to the CFIA Food Safety Investigation Program work specifications.

4.2 If necessary, samples are ground up and then homogenised. A sub-sample of about 500 grams is taken and stored before analysis.

5.0 Extraction

Recommended procedure:

5.1 Wheat, Oats, and Oat Bran Samples

5.1.1 Weigh 2 g sample into a stomacher bag and extract with 8 mL acetonitrile / water (80 / 20) extraction solution.

5.1.2 For a matrix spike, add appropriate volumes of Master Mix 1 and Master Mix 2 solutions into the sample. For example, addition of 100 µL of Master Mix 1 and 100 µL of Master Mix 2 into a 2 g sample gives the following spike levels.

20 ng/g:	aflatoxins, sterigmatocystin, OTA and cyclopiazonic acid
200 ng/g:	zearalenone, α-zearalenol, β-zearalenol, fumonisins, trichothecenes (DON, NIV, FUS-X, ADONs, NEO, DAS, HT-2, and T-2) and ergot alkaloids

5.1.3 To the sample / extraction solution mix, add 8 µL of ascorbic acid (1 mg/mL) to protect against degradation of CPA.

5.1.4 Extract the sample for 2 minutes at normal speed in a Seward Stomacher 80 laboratory blender.

5.1.5 Decant the extract into a 15 mL polypropylene centrifuge tube and centrifuge for 10 minutes at 3,000 rcf, 10°C.

5.1.6 Dilute 250 µL of supernatant with 750 µL Milli-Q water, vortex-mix and syringe-filter through a 0.2 µm PTFE filter.

5.1.7 Analyse by LC-MS/MS.

5.2 Wheat Bran Samples

5.2.1 The extraction procedure is the same as in section 5.1 except for the following differences. Use 12 mL acetonitrile / water (80 / 20) extraction solution to extract the 2 g sample. Add 12 µL of ascorbic acid (1 mg/mL) to the sample / extraction solution mix to stabilise CPA.

5.3 Corn Samples

5.3.1 Weigh 2 g sample into a stomacher bag and extract with 5 mL acetonitrile / water (80 / 20) extraction solution.

5.3.2 For a matrix spike, add appropriate volumes of Master Mix 1 and Master Mix 2 solutions into the sample. For example, addition of 100 µL of Master Mix 1 and 100 µL of Master Mix 2 into a 2 g sample gives the following spike levels.

20 ng/g:	aflatoxins, sterigmatocystin, OTA and cyclopiazonic acid
200 ng/g:	zearalenone, α-zearalenol, β-zearalenol, fumonisins, trichothecenes (DON, NIV, FUS-X, ADONs, NEO, DAS, HT-2, and T-2), and ergot alkaloids

5.3.3 To the sample / extraction solution mix, add 8 µL of ascorbic acid (1 mg/mL).

5.3.4 Extract the sample for 2 minutes at normal speed in a Seward Stomacher 80 laboratory blender.

5.3.5 Add 3 mL Milli-Q water to the sample / extraction solution mix resulting in a 50 / 50 acetonitrile / water ratio.

5.3.6 Extract the sample again for 2 minutes at normal speed in a Seward Stomacher 80 laboratory blender.

5.3.7 Decant the extract into a 15 mL polypropylene centrifuge tube and centrifuge for 10 minutes at 3,000 rcf, 10°C.

5.3.8 Dilute 250 µL of supernatant with 750 µL Milli-Q water, vortex-mix and syringe-filter through a 0.2 µm PTFE filter.

5.3.9 Analyse by LC-MS/MS.

6.0 LC-MS/MS Analysis

6.1 LC-MS/MS System consisting of:
- AB 4000 QTRAP Mass spectrometer, or equivalent
- Agilent 1200 series HPLC, or equivalent

6.2 Recommended HPLC Conditions

6.2.1 General HPLC Conditions

Column	Alltima HP C18 minicolumn (5 µm particle size, 7.5 x 2.1 mm I.D.) or equivalent	
Mobile phases	A: Water with 0.1% formic acid	
	B: Methanol with 0.1% formic acid	
Needle wash	Acetonitrile / Methanol / water / acetic acid: 40/50/10/1 or other appropriate compositions	
Gradient (9 min)	0 to 1.0 min	1% B
	1.0 to 4.5 min	linear gradient to 99% B
	4.5 min to 7.5 min	99% B
	7.5 min to 7.6 min	returned to 1% B
	7.6 min to 9 min	Re-equilibration at 1% B
	Total time	9 min
Flow Rate	1000 µL / min	
Injection volume	5 µL	
Column temperature	10°C	
Auto sampler temperature	4°C	

6.2.2 HPLC Conditions for Ammonia Injection

Ammonia solution is injected after every injection of standard or sample in order to eliminate carry-over of CPA and prolong the life of the column. Except for the following modifications to the HPLC gradient, other conditions are kept the same as above.

Gradient (4.5 min)	Initial	1% B
	0 to 0.1 min	linear gradient to 99% B
	0.1 to 3.0 min	99% B
	3.0 min to 3.1 min	returned to 1% B
	3.1 min to 4.5 min	Re-equilibration at 1% B
	Total time	4.5 min

6.3 Recommended MS/MS Conditions

Scheduled multiple reaction monitoring (sMRM) with a detection window of 42 seconds for each compound is used to determine each mycotoxin. Please see Appendix C for a summary of MS/MS conditions and chromatographic retention times for the 24 mycotoxins.

Ion Source	Turbo Spray
Polarity	Positive
Scan Type	MRM
Resolution Q1	Unit
Resolution Q3	Unit
CUR	20 L/min
IS	5500 V
CAD	12 V
TEM	600℃
GS1	55 L/min
GS2	45 L/min
EP	10 V
Interface Heater	Off

7.0 Performance

Separation	baseline
Repeatability	<20%
Recovery	50 -150%
Limit of detection (LOD)	See Appendix A
Limit of quantitation (LOQ)	See Appendix A
Reporting limit	Same as LOD
Uncertainty	See Appendix D

7.1 The uncertainty estimates were determined using data obtained from spike recoveries (n=2) at 20 ng/g, 200 ng/g or 250 ng/g. The internal relative repeatability standard deviations were calculated and multiplied by a coverage factor of 2 to give the expanded uncertainties. Please see Appendix D for details.

8.0 Calculations

8.1 Calculations are carried out using Analyst software.

8.2 Fill in the standard concentrations and sample dilution factors into Analyst software. See Table C for dilution factors.

Table C. Dilution Factors Used in Mycotoxin Calculation.

Commodity	*Sample Weight (g)*	*Volume of Extraction Solution (mL)*	*Dilution*	*Overall Dilution Factor*
Wheat, corn, oats, oat bran	2	8	1 + 3	16
Wheat bran	2	12	1 + 3	24

9.0 Confirmation

9.1 If the identity of an observed quantifier peak is in question: Calculate the peak area ratio of qualifier ion to the quantifier ion. The ratio should be within ± 20% of the means of the ion ratios of the standards.

10.0 Quality Control

10.1 Spiked Recoveries - In general, recoveries of the mycotoxins should be between 50 and 150% in this screen method. The exceptions are fumonisins, which are recovered at <50% in wheat flour, oat flour, wheat bran and oat bran.

10.2 Worksheet Details

Template	Template – Multimycotoxin Worksheet v1.0, RDIMS document #3029659
Title	Multimycotoxin YYMMDDinit, where 'init' are the initials of the analyst
Organisation	RAU6A
Document Type	TECH
File Number	35 35201
Notes/Tracking Number	worksheet-chem
Security Access	Burnaby Lab-All Staff (Deselect 'Edit Profile') Burnaby Lab QAOs - Full Access

10.3 Method Implementation

A multi-point calibration curve is used to quantify samples. A negative control sample and a sample spiked with all mycotoxins (n=2) are extracted with every batch to determine spike recovery.

11.0 Reporting of DON Results

11.1 Report results greater than the reporting limit in ppb (ng/g). Report results below the reporting limit as <0 in LSTS, which shows up as "not detected at the reporting limit" in the RoA.

11.2 The following is based on Health Canada guidelines for DON in wheat. See http://www.hc-sc.gc.ca/fn-an/securit/chem-chim/contaminants-guidelines-directives-eng.php for details. Also refer to the CFIA Imported and Manufactured Foods Division Policy FDA 4-2 draft document, "Deoxynivalenol Limits in Foods" (RDIMS #1637722).

11.3 Results are to be interpreted as follows:

Table D. Deoxynivalenol Reporting Limits and Assessments.

Matrix	*Limit*	*≤ Limit Assessment*	*> Limit Assessment*
Hard (durum) wheat flour	0.75 µg/g	Satisfactory	Investigative

Matrix	*Limit*	*≤ Limit Assessment*	*> Limit Assessment*
Uncleaned soft wheat flour for use in non-staple foods	2 µg/g	Satisfactory	Investigative
Uncleaned soft wheat flour for use in infant foods	1 µg/g	Satisfactory	Investigative
Unknown	0.75 µg/g	Satisfactory	No Assessment

11.4 For all unknown commodities that exceed the limit, "no assessment" is to be entered in the comments field. Results may still be subject to further assessment and review by Operations and Programs staff.

11.5 For proficiency test (PT) schemes, report results according to instructions received with PT samples.

12.0 Reporting of OTA Results

12.1 Health Canada is developing guidelines for maximum limits of OTA in various food commodities. The proposed maximum limits are listed in Table E below.

Table E. Proposed Maximum Limits for OTA in Various Food Commodities.

Commodity	*Proposed Limit*
Raw cereal grains	5 ng OTA/g
Directly consumed grain	3 ng OTA/g
Derived cereal products (flour)	3 ng OTA/g
Derived cereal products (wheat bran)	7 ng OTA/g
Grape juice and related products	2 ng OTA/g
Baby foods	0.5 ng OTA/g
Dietary foods for special medicinal purpose intended for infants	0.5 ng OTA/g

12.2 Report results greater than the reporting limit in ppb (ng/g), to one decimal place. Report results below the reporting limit as <0 in LSTS, which shows up as "not detected at reporting limit" in the RoA.

12.3 For proficiency test (PT) schemes, report results according to instructions received with PT samples.

13.0 Reporting of Aflatoxin Results

13.1 Report results for each individual aflatoxin as well as total aflatoxins, which is determined by adding the results of the individual aflatoxins together.

13.2 Results are to be interpreted as follows:

- Total aflatoxin below detection limit is reported as "Not Detected". Enter "<0" in the result entry field to have LSTS automatically insert the phrase "not detected at the reporting limit" in the Report of Analysis.
- Total aflatoxin less than or equal to 15 ppb - Satisfactory
- Total aflatoxin between 15-23 ppb - Investigative

- Total aflatoxin greater than 23 ppb - Unsatisfactory, in violation of Food and Drug Regulations B.01.046(1)(n) for nut and nut products only. Any other commodity is interpreted as investigative.

13.3 For proficiency test (PT) schemes, report results according to instructions received with PT samples.

14.0 Reporting Results of Other Mycotoxins

14.1 Report results greater than the reporting limit in ppb (ng/g), to one decimal place. Report results below the reporting limit as <0 in LSTS, which shows up as "not detected at reporting limit" in the RoA.

14.2 For proficiency test (PT) schemes, report results according to instructions received with PT samples.

Appendix A: LOD and LOQ Summary

Compound	*Calibration Range (ng/g)*	*LOD (ng/g)*	*LOQ (ng/g)*
AFB1	2-64	3	9
AFB2		3	9
AFG1		3	9
AFG2		4	12
STE		3	9
OTA		3	9
CPA		2	6
T-2	10-640	15	45
HT-2		17	60
DAS		10	30
NEO		10	30
FB1		15	45
FB2		15	45
FB3		15	45
Ergocristine		15	45
Ergocryptine		15	45
Ergosine		15	45
DON	20-640	50	150
ADONs		50	150
FUS-X		20	60
ZEA		20	60
α-ZOL		20	60
β-ZOL		20	60
NIV	40-640	60	180

Appendix B: Maximum Absorbances (λ_{max}) and Molar Extinction Coefficients (ε)

Mycotoxin	*Molecular Weight (g/mol)*	*Solvent*	λ_{max}	*Molar Extinction Coefficient (ε)*
15-acetyldeoxynivalenol	338.35	Acetonitrile	220	6935
Aflatoxin B1	312.3	Acetonitrile	350	20700
Aflatoxin B2	314.3	Acetonitrile	350	22500
Aflatoxin G1	328.3	Acetonitrile	350	17600
Aflatoxin G2	330.3	Acetonitrile	350	18900
Cyclopiazonic Acid	336.4	Methanol	224	39810
Deoxynivalenol	296.3	Acetonitrile	218	6400
Diacetoxyscirpenol	366.4	Methanol	202	2487
HT-2 Toxin	424.5	Methanol	201	4001
Neosolaniol	382.4	Methanol	202	2644
Ochratoxin A	403.8	Toluene, 1% Acetic Acid	333	5440
T-2 Toxin	466.5	Methanol	202	4022
Zearalenone	318.4	Methanol	236	29200

Appendix C: Summary of MS/MS Conditions and Chromatographic Retention Times

Mycotoxin	*RT (min)*	*Transition* [Note 1]	*Precursor Ion $[M + H]^+$*	*Product Ion $[M + H]^+$*	*DP (volts)*	*CE (volts)*	*CXP (volts)*
AcDONs	2.33	1	339.1	231.1	50	19	40
	2.34	2		279.1	81	13	14
AFB_1	3.02	1	313	128	100	95	5
	3.02	2		285	100	35	17
AFB_2	2.94	1	315	259	80	45	14
	2.94	2		287	80	35	16
AFG_1	2.85	1	329	200	90	58	10
	2.85	2		243	90	40	5
AFG_2	2.77	1	331	245	70	35	7
	2.77	2		313	70	45	16
α-ZOL	3.65	1	321.2	285.01	51	15	18
	3.65	2		267.1	41	19	16
β-ZOL	3.47	1	321.2	285	51	15	18
	3.47	2		267	41	19	16
CPA	3.85	1	337.2	196	96	33	10
	3.85	2		182.1	96	27	8
DAS	2.9	1	384.2[Note 2]	307	51	17	16
	2.9	2		247.1	51	21	14
DON	0.66	1	297	249.2	36	14	14
	0.66	2		203.1	38	22	10
Ergocristine	3.17	1	610.3	223.1	81	57	14
	3.17	2		268.1	81	35	12
Ergocryptine	3.11	1	576.3	223.1	76	55	10
	3.11	2		268.1	76	37	14
Ergosine	2.95	1	548.3	208.1	61	57	14
	2.95	2		223.1	61	47	12
FB_1	3.56	1	722.4	352.4	91	55	9.6
	3.56	2		334.4	91	55	9.6
FB_2	3.84	1	706.5	336.4	96	53	20
	3.84	2		318.4	96	49	22
FB_3	3.74	1	706.51	336.41	96	53	20
	3.74	2		318.41	96	49	22
FUS-X	2.00	1	355.1	247.3	76	13	12
	2.00	2		228.9	76	21	14
HT-2	3.23	1	442.2[Note 2]	263.2	46	19	14
	3.23	2		215.1	46	19	14
NEO	2.22	1	400.2[Note 2]	305.2	51	17	16
	2.22	2		245.1	51	17	12
NIV	0.28	1	313.1	175.1	76	23	8
	0.28	2		137.1	76	19	8
OTA	3.72	1	404.1	238.9	66	33	16
	3.72	2		221.1	66	51	10
STE	3.67	1	325.1	281	91	51	14
	3.67	2		252.9	91	61	12
T-2	3.44	1	484.3	305.2	36	19	6
	3.44	2		244.9	36	19	12
ZEA	3.65	1	319.2	283.1	71	19	14
	3.65	2		187	71	29	10

[Note 1] Transition 1 is the quantifier ion and transition 2 is the qualifier ion.
[Note 2] $[M + NH_4]^+$

Appendix D: Measurement Uncertainty Estimate Based on Repeatability Data

Mycotoxin	*Concentration (ng/g)*	*Expanded Uncertainty (±)*
AFB_1	20	2.1
AFB_2	20	2.1
AFG_1	20	3.2
AFG_2	20	3.5
CPA	20	1.9
OTA	20	2.5
STE	20	2.1
AcDONs	200	26
DON	200	21
NIV	200	37
DAS	200	11
FB_1	200	31
FB_2	200	22
FB_3	200	14
FUS-X	200	16
HT-2	200	25
T-2	200	21
NEO	200	17
α-ZOL	200	29
β-ZOL	200	47
ZEA	200	36
Ergocristine	250	35
Ergocryptine	250	37
Ergosine	250	34

CVDR-M-4014.02

A Determinative and Confirmatory Method for Phthalate Esters in Foods by LC-MS/MS

Canadian Food Inspection Agency
Saskatoon Laboratory
Centre for Veterinary Drug Residues
116 Veterinary Road
Saskatoon, SK S7N 2R3

References:

"*Taiwanese method*" – *Analysis of Phthalate Esters in Foods*
FDA translations of "Taiwanese method"
CFIA-FDA joint validation, August 2011 Summary (RDIMS 3338366)

1. Scope: This test method is suitable for the determinative and confirmatory testing of the phthalate esters benzyl butyl phthalate (BBP), di-butyl phthalate (DBP), di-ethyl-hexyl phthalate (DEHP), di-n-octyl phthalate (DNOP), di-iso-nonyl phthalate (DINP), and di-iso-decyl phthalate (DIDP) in infant formula (powder, liquid), infant cereals (dry), processed baby foods (fruit and vegetables), bottled water, jams, jellies, juices, teas and powders. The method's detection limit is 0.5 µg/g, sample equivalents and has a CFIA guidance level of 1 µg/g, sample equivalents.

2. Principle: One gram of sample is extracted with 50 mL of methanol with sonication followed with centrifugation. A sub-aliquot of the extract is held at -20°C overnight extract to assist with de-fatting of the extract. The extract is centrifuged the next day at -5°C and is injected onto an LC-MS/MS system for analysis.

3. Apparatus:

3.1 Notes:

3.1.1 Suppliers listed for reference only. Other brands of equal performance may be substituted unless noted otherwise.

3.1.2 All volumetric glassware used throughout this method is Class A.

3.1.3 **Critical Procedure: Due to the prevalence of phthalates as a routine contaminant from plastics, wherever possible use glass-based apparatus only; rinse items noted with an asterix (*) with methanol and dry before use.**

3.2 Centrifuge (capable of 6000xg), Beckman Avanti Centrifuge, JS-5.3 rotor with 50 mL tube adaptors. Beckman Allegra Centrifuge, GH 3.8 rotor with 15 mL tube carrier

3.3 Glass volumetric flask 10 mL, 25 mL, 50 mL, 100 mL* see note 3.1.3

3.4 Glass bottle, Teflon lined/foil lined cap: 100-250mL* see note 3.1.3

3.4.1 These are required for storage of blank material (bulk pools) and for confirmed positive samples in which the original container cannot be re-sealed post sub-sampling. **Samples are to be retained in their original packaging whenever possible.**

3.5 Glass graduated cylinder, 100 mL with glass stoppers. The cylinders and stoppers are to be baked overnight at approximately 200°C prior to use.

3.6 LC column: ACQUITY BEH Phenyl-hexyl or Waters CSH Phenyl-hexyl, 1.7 µm, inner diameter of 2.1 mm × 50 mm.

3.7 LC-MS/MS system: Waters UPLC with a TQD MS/MS detector and Masslynx software (or alternate LC-MS/MS system providing adequate sensitivity).

3.8 LC vials, Teflon lined caps. The vials are to be baked overnight at approximately 200°C prior to use.

3.9 Pasteur pipettes

3.10 Polypropylene conical tubes, 15mL and 50mL sizes, Falcon brand. No substitutions: If considering using another brand, evaluate for contributions of phthalates prior to use by concentrating an aliquot of methanol used to rinse the tube and analysing the concentrate for phthalate residues.

3.11 Sonicator

3.12 Scintillation vials (20 mL) with foil lined lids. Bake at 200°C (typically overnight) prior to use.

3.13 Syringes, glass barrel, gas tight. 10, 25, 50, 100 and 2500 µL

3.14 Vortex Mixer

4. Reagents:

4.1 Notes:

4.1.1 Manufacturers listed for reference only. Other brands of equivalent (or better) grade may be substituted.

4.1.2 All water used throughout the method was purified by reverse osmosis followed by deionization, adsorption and filtration.

4.1.3 Preparation instructions are provided for guidance purposes only and, unless noted otherwise, the volume required can be adjusted to allow for more or less solution as required. Preparation details are to be recorded in the reagent preparation log.

4.2 Formic Acid, concentrated (EMD chemicals, Gibbstone, NJ, USA)

4.3 Formic Acid, 0.1% in water: Add 1.0 mL formic acid to 800 mL water in a 1L volumetric flask. Mix and bring to volume with water.

4.4 Methanol, distilled in glass (Caledon).

5. Standard Solutions:

5.1 Notes:

5.1.1 In addition to following the safety procedures outlined in the Agency's Laboratory Safety Manual and the Saskatoon Laboratory's Safe Work Practices and Handling, Storage and Disposal of Chemicals and Hazardous Waste, the analyst must review the CVDR Job Hazard Analysis (JHA) for preparation of standards and relevant Material Safety Data Sheets (MSDS's). **Avoid direct contact with analytical standards. Wear disposable nitrile gloves, lab coat and protective eyewear.**

5.1.2 Standard reference material required is listed below. For current supplier details, contact the Centre's Proficiency Testing Unit.

5.1.2.1 Benzyl butyl phthalate (BBP, CAS# 85-68-7), Dibutyl phthalate (DBP, CAS # 84-74-2), Di(2-ethylhexyl) phthalate (DEHP, CAS# 117-81-7), Di-n-octyl phthalate (DNOP, CAS# 117-84-0), Di isononyl phthalate (DINP, CAS# 68515-48-0) were purchased

through Sigma Aldrich. Di isodecyl phthalate (DIDP, CAS#26761-40-0) was purchased from Accustandard, Inc.

5.1.2.2 Benzyl butyl phthalate-d4 (BBP-d4, CAS# 93951-88-3), Dibutyl phthalate-d4 (DBP-d4, CAS# 93952-11-5), Di(2-ethylhexyl) phthalate-d4, (DEHP-d4, CAS# 93951-87-2), Di-n-octyl phthalate-d4 (DNOP-d4, CAS# 93952-13-7) – deuterated standards – were purchased from Cambridge Isotopes. Bis (7-methyl-1-octyl) phthalate-d4 (DINP-d4, CAS# 1332965-90-8), Di-isodecyl-phthalate-d4 (DIDP-d4, CAS# 1346604-79-2) were purchased from Canadian Isotopes.

5.1.3 Standard preparation instructions are provided for guidance purposes only and, unless noted otherwise, the volume required can be adjusted to allow for more or less solution as required. Preparation details are to be recorded in the standards preparation log.

5.1.4 To determine the weight of the standard required, the analyst must know the chemical form (hydrochloride, sodium salt, etc.) and assayed purity of the analytical standard material, taking both into account when determining the actual amount to weigh for a given concentration.

Example:

$$\text{Corrected mass} = \text{Target Mass} \times \frac{100}{\text{Purity, \%'age}} \times \frac{\text{Molecular Weight (chemical form, salt)}}{\text{Molecular Weight (free base)}}$$

5.1.5 The *actual* stock standard concentration may vary slightly from the *target* concentration. In that event, the amount required to prepare a given concentration of a working standard solution will need to be adjusted accordingly to ensure that the working solution concentration is maintained at the target value.

5.2 Stock Standard Solutions (1000 µg/mL, approx)

5.2.1 Weigh 0.05 g (corrected for purity, etc as appropriate) of each of the phthalate and deuterated standards into separate 50 mL glass volumetric flasks, dissolve and bring to volume with methanol. Record the exact amount of reference material used and the corrected concentration. Store at 4° C in pre-baked scintillation vials. Expires, 1 year (conditional).

5.3 Mixed Solutions, typical concentrations, prepare as required. The dilutions required may vary depending on the source of the parent solution. Record exact preparation details in the standards preparation log.

5.3.1 100 µg/mL mixed intermediate Phthalate solution: Transfer 1mL of each of the 1000 µg/mL non-deuterated stock phthalate standards into a 10mL volumetric flask. Make to volume with methanol. Store at 4° C in pre-baked scintillation vials. Expires, 1 month.

5.3.2 25 µg/mL mixed intermediate Phthalate solutions: Transfer 0.250 mL of each of the 1000 µg/mL Phthalate stock solutions into a 10 mL volumetric. Make to volume with methanol. Alternatively prepare an appropriate dilution of the 100 µg/mL mixed intermediate solution. Store at 4° C in pre-baked scintillation vials. Expires 1 month.

5.3.3 2.5 µg/mL mixed working Phthalate solution – required only for the deuterated standards: Transfer 500 µL of the 25 µg/mL Intermediate phthalate solution into a 5mL volumetric flask. Store at 4° C in pre-baked scintillation vials. Make to volume with methanol. Expires, 1 month.

5.3.4 1 µg/mL mixed working Phthalate solution. Transfer 100 µL of the 100 µg/mL mixed intermediate Phthalate solution into a 10mL volumetric flask. Make to volume with methanol. Store at 4° C in pre-baked scintillation vials. Expires, 1 month.

5.3.5 Repeat, steps 5.3.1 through 5.3.4 for the deuterated standards, preparing mixed solutions of those standards at each of 100 µg/mL, 25 µg/mL, 2.5 µg/mL and 1 µg/mL.

6. Extraction:

6.1 Notes:

6.1.1 In addition to following the safety procedures outlined in the Agency's Laboratory Safety Manual and the Saskatoon Laboratory's Safe Work Practices and Handling, Storage and Disposal of Chemicals and Hazardous Waste, the analyst must review the relevant Job Hazard Analyses (JHAs) and Material Safety Data Sheets (MSDSs).

6.1.2 **CRITICAL CONTROL POINT: Unless otherwise noted, use glass-based labware during the extraction to minimize the risk of background phthalate contamination.**

6.1.3 Where a suitable blank is not available and/or the performance in the noted matrix has not been fully established, include, in addition to the unknown sample, that same sample, fortified at the level of interest.

6.1.4 Generally all samples are first set up in a screening run.

6.1.5 Determinative analyses are required for suspects > 1.0 µg/g, sample equivalents, for all suspect positive residues except DBP, which is flagged for repeat if the suspect level is > ½ LOD. Samples being set up for a determinative analysis for DBP require the use of glassware which has been baked overnight at >100°C.

6.1.6 The spikes set up for a respective determinative run (6.3) are provided for guidance purposes. Determinative run requirements are established in consult with the program chemist and will vary depending on the analyte observed and the matrix of interest.

6.2 Screening run samples:

6.2.1 Add 1.0 ± 0.01 g of each test sample into separate 50 mL polypropylene tubes or baked graduated cylinder, as applicable.

6.2.1.1 For pureed vegetables/fruits, jams, jellies – mix to blend and select aliquot from the jelly of the sample, excluding the seeds where possible. The sample can be heated to 38°C to facilitate easier subsampling if the jelly/jam is difficult to weigh (include a record of the need to heat the sample if this is done). Record the cooled (to room temperature) sample weight.

6.2.1.2 For powders – select a uniform subsample. If blending is required or a composite sample is taken to obtain the required test sample weight, record the blending details.

6.2.1.3 For liquids (bottled waters/beverages/juices/formula, syrups) – ensure sample is well mixed prior to subsampling.

6.2.1.4 Non-routine samples – contact the program supervisor for sampling details. Typically, in addition to the non-routine sample analysed as a test sample, that same material is set up as a matrix-fortified spike at 1.0 µg/g tissue equivalents (TE).

6.2.1.5 Refrigerate excess test material/sample, subsampling into a glass container/jar (teflon-lined lid) in the event the original container cannot be re-sealed.

6.2.2 Screening standards: Prepare chemical standards at 10 ng/mL and 20 ng/mL, in vial (0.5 and 1.0 µg/g, tissue equivalents, sample equivalents). Add 5µL and 10 µL of the 1 µg/mL mixed Phthalate solution into separate LC vials containing 500 µL methanol.

6.2.3 Quality Control Samples: Weigh out three 1.0 ± 0.01 g aliquots of blank material, selecting a blank type (jam, jelly, syrup, etc) that is representative of the majority of test samples intended for analysis into separate 50 mL polypropylene tubes or baked glass graduated cylinders, as applicable.

6.2.3.1 Add 20 and 40 µL of the 25 µg/mL mixed intermediate Phthalate's solution to two of the three blank tissue aliquots. This will provide matrix-fortified calibration standards of 0.5 and 1.0 µg/g, sample equivalents. The third blank sample will serve as the negative control sample and the source of the extract required to prepare the matrix-matched over-spikes which will be used to evaluate recovery (step 6.11).

6.2.4 For mixed commodity type runs, where method performance vary between matrices, include at least one additional quality control sample in the "other" matrix type(s) at 1.0 µg/g, sample equivalents (40 µL of the 25 µg/mL mixed intermediate Phthalates solution).

6.2.5 Include a reagent blank, non-spiked, processed through the entire run to monitor background phthalate levels.

6.3 Determinative run samples (targetted analyses):

6.3.1 Prepare the chemical calibration at levels comparable to the matrix fortified standards (see 6.3.3).

6.3.2 Quality Control samples:

6.3.2.1 Weigh three 1.0 g aliquots from a phthalate free blank material. Add 20µL and 40 µL of the 25 µg/mL mixed intermediate Phthalates solution to one of the two samples, to serve as the LOD spike (0.5 µg/g, sample equivalents) and the positive control, 1.0 µg/g, sample equivalents. The third blank will serve as the negative control as well as the source for the matrix-matched recovery samples.

6.3.2.2 Include a reagent blank, non-spiked, processed through the entire run.

6.3.3 Weigh out the suspect sample, 1.0 g aliquot(s) into a 50 mL polypropylene tube or a glass graduated cylinder (baked), as appropriate. If the suspect level of phthalates in the test sample is especially high, a combination of a reduced weight and post extraction dilutions with methanol can be used to achieve a result within the 10-40 ng/mL in vial range.

6.3.3.1 For pureed vegetables/fruits, jams, jellies, liquids – weigh one sample. Prepare an external matrix fortified curve using a matrix comparable to the suspect positive, including levels which bracket the suspect analyte level (typical analytical range 10 – 200 ng/mL in vial).

6.3.3.2 For powders – weigh in quadruplicate. Spike two of the four samples at a level approximately 4X the suspect analyte level. Apply standards addition approach to quantitation.

6.3.3.3 Non-routine samples – contact the program supervisor for determinative analysis details.

6.4 To each sample (test sample, calibration set and positive QC('s)), **excluding the negative control sample,** add 20 µL of the 100 µg/mL mixed deuterated standard solution. Vortex briefly and allow to sit at room temperature for 15 minutes.

6.5 Using the graduations on the polypropylene tubes and/or graduated cylinder as a marker, add methanol up to the 50mL marker, cap/cover and sonicate for 30 minutes. For the samples set up in graduated cylinders, approximately 5 mL of the sample is taken from the graduate cylinder and transferred to a (baked) 15 mL glass disposable centrifuge tube (foil lined cap).

6.6 Centrifuge the samples at room temperature for 10 minutes.

6.6.1 The samples in the 50 mL Falcon tubes are centrifuged at 3300 x g.

6.6.2 The samples in the 15 mL glass centrifuge tubes are centrifuged at 1160 x g.

6.7 Sub-sample approximately 3 mL of the supernatant from each of the samples into 15mL polypropylene disposable centrifuge tubes or a glass centrifuge tube as applicable. Place all tubes overnight in the freezer (ca. -20°C).

Note: This will precipitate fatty residues that have been carried through the extraction. While the fat content does not impact the method's analytical performance, it reduces the lifetime of the analytical column. This step can be omitted upon consultation with the program supervisor, proceeding directly to transfer to the LC vials.

6.8 The next morning, centrifuge the samples at -5°C for 15 minutes.
- 6.8.1 The samples in the 50 mL Falcon tubes are at 6000 x g
- 6.8.2 The samples in the 15 mL glass centrifuge tubes are centrifuged at 1160 x g.

6.9 Keeping those samples as cold as possible (on ice for example), sub-sample approximately 500µL of the supernatant from each of the samples into LC vials for analysis by LC-MS/MS.

Note: Minimize the time the centrifuged extracts are exposed to room temperature, otherwise the fatty residues will go back into solution.

6.10 For the negative control sample and the suspect positive (non-spiked aliquot), prepare matrix matched recovery samples. Dispense 500 µL into an LC vial. Spike as noted below, preparing matrix matched samples that can be used to determine % recovery.
- 6.10.1 Spike into 500 µL of the negative control extract, 5 µL of the 1 µg/mL mixed Phthalate solution (10 ng/mL in-vial) – for comparison to the LOD quality control spike.
- 6.10.2 Spike into 500 µL of the negative control extract, 10 µL of the 1 µg/mL mixed Phthalate solution (20 ng/mL in-vial) – for comparison to the 1 ppm, sample equivalents, positive quality control spike.
- 6.10.3 To each of the above, add 8 µL of the 2.5 µg/mL deuterated Phthalate solution.

7. Instrumental Analysis:

7.1. Notes:
- 7.1.1. Parameters are provided for guidance and may need to be optimized
- 7.1.2. Clean/ replace the sample cone of the MS ionization source if a decrease in instrument performance has been observed in previous runs.

7.2. LC parameters
- 7.2.1. The parameters provided are based on a Waters-UPLC system (UPLC-TQD MS/MS) configuration.

Note: The shallow gradient is required to minimize the elution of phthalates inherent to the LC system while still allowing for optimal retention of the injected analytes.

Column temperature: 60°C
Sample chamber temperature: 5°C
Injection volume: 3 µL
Run time: 2.95 min
Solvents: A – Methanol; B – 0.1% Formic Acid in Water
Column: BEH Phenyl-hexyl, 2.1 x 50 mm, 1.7 µm

Pump gradient

Time (min)	Flow Rate	%A	%B	Curve
Initial	0.858	77	23	
0.12	0.858	77	23	6
0.15	0.700	77	23	6
1.10	0.700	81	19	6
1.27	0.700	84	16	6
1.65	0.700	86.5	13.5	6
1.75	1.000	99	1	6
2.45	1.000	99	1	6
2.50	1.000	77	23	6

7.3. MS/MS Parameters

7.3.1. The settings proposed are typical for a Waters UPLC-TQD MS/MS system and may vary depending on optimization of the tune settings.

Capillary voltage: 0.85 kV
Ion source temperature: 120 °C
Desolvation temperature: 500 °C
Cone gas flow rate: 60 L/hr
Desolvation flow rate: 1100 L hr
Detection mode: multiple reaction monitoring (MRM), ES+
Monitored ion pair masses (nominal values only), cone voltage and collision energy as follows:

Compound	Monitored Reaction Precursor>Product Ion	Cone Voltage	Collision Energy
BBP	313 > 149[(1)]	17	11
	313 > 205*	17	7
	313 > 239	optional, evaluate as required	
DBP	279 > 149*	20	14
	279 > 205[(1)]	20	7

DEHP	391 > 149*	19	20
	391 > 167(1)	19	14
	391 > 71	optional, evaluate as required	
	391 > 113	optional, evaluate as required	
	391 > 279	optional, evaluate as required	
DNOP	391 > 149(1)	18	12
	391 > 261*	18	10
	391 > 121	optional, evaluate as required	
DINP	419 > 149*	15	26
	419 > 275(1)	15	12
	419 > 71	optional, evaluate as required	
	419 > 127	optional, evaluate as required	
	419 > 167	optional, evaluate as required	
	419 > 293	optional, evaluate as required	
DIDP	447 > 141*	22	11
	447 > 149	22	25
	447 > 289(1)	22	10
	447 > 167	optional, evaluate as required	
	447 > 307	optional, evaluate as required	
	447 > 99	optional, evaluate as required	
DBP-D4	283 > 153*	20	14
BBP-D4	317 > 91*	17	11
DEHP-D4	395 > 153*	30	30
DNOP-D4	395 > 153*	18	12
DINP-D4	453 > 153	15	22

(1) Confirmatory transitions

* Quantitation mass

Those masses not flagged with either an (*) or a (1) are provided for references purposes only. Their use is discretionary and prior to use would require review/optimization.

7.4.1 Suitability criteria: Analyte elution order is as noted in Appendix I. Analytes are to be detected and the required product ions for the respective analytes present; DEHP and DNOP are to be approximately 90% resolved. If resolution between DNOP and DEHP appears to be failing, this is an indicator that the column should be reviewed for replacement. The confirmatory ratio data, when reviewed for the known positive samples (matrix fortified/matched samples), are to be comparable to one another within method

specifications (see section 9). The retention times of the respective analytes for fortified samples are to fall within 2.5% of one another.

7.4.1 If the criteria are not met, consult with the responsible supervisor. Investigate and identify probable cause, documenting this information with the run.

8. Calculations:

8.1. For samples in which matrix effect has been demonstrated to have minimal impact on the returned results, the phthalate ester content (µg/g, sample equivalents) in the sample can be calculated as follows:

Using the calibration standards (chemical or matrix fortified), linear regression is done using the area response ratio data of the respective analytes relative to the internal standard response, quantitation masses (as y), versus the concentration of the analytes, (as x).

Phthalate ester content (ng/mL, in vial), $x = (y-b)/m$

Where,
y = area, quantitation mass
b = y-intercept
m = slope

Phthalate ester content (µg/g, sample equivalents) in sample =

$$\text{In-vial result (ng/mL)} \times 50\text{ mL} \times \text{dilution factor (if any)} \times \frac{\text{Sample Weight (theoretical) g}}{\text{Actual Sample Weight}} \times \frac{1\ \mu g}{1000\ ng}$$

8.2 The standards addition approach for quantitation can be applied to any analyte but is typically done for those samples where matrix effects are suspected to impact the ability to use an external chemical or matrix-fortified (but alternate blank) calibration set for quantitation of the analyte response. Linear regression is done using the response ratio of the area of the respective analyte response relative to the analyte-specific internal standard response, quantitation masses (as y), versus the concentration of the analytes, (as x); the non-fortified sample is assumed to be "0", x value.

The actual level of analyte in the non-fortified sample is the (absolute) value at which the linear regression line intercepts with the x-axis. This value is also obtained by dividing the y-intercept (b) by the slope (m),

Phthalate ester content (concentration units, as plotted with "x", typically presented as in vial, ng/mL) = b/m.

To correct in vial result (ng/mL) to amount in sample:
Phthalate ester content (µg/g, sample equivalents) in sample =

$$\text{In-vial result (ng/mL)} \times 50\text{ mL} \times \text{dilution factor (if any)} \times \frac{\text{Sample Weight (theoretical) g}}{\text{Actual Sample Weight}} \times \frac{1\ \mu g}{1000\ ng}$$

8.3. Recovery Samples. Using the area count responses obtained for the quantitation mass for each of the analytes, compare the matrix fortified spike results (1.0 µg/g, sample equivalents) to those obtained for the matrix-matched spike (20 ng/mL, in vial). To extend this review to include a review of the matrix effect, compare the matrix-matched spike response to the chemical standard set up at a comparable level.

Recovery data for analyst spiked liquid infant formula, water, jams, jellies, juices, syrups and teas can be calculated from either a chemical standard or a matrix-matched sample - ie minimal matrix effect in those sample types.

Recovery data for analyst spiked powders (including formula, baby cereal, others) as well as the processed baby foods should generally be calculated relative to a matrix-matched (over-spiked) blank. These product types have demonstrated matrix effect some of the analytes, however this has varied from run to run (preliminary data).

9. Confirmation:

9.1. Suspect positives for DBP, if greater than ½ the DBP LOD response, are considered DBP suspect and will require repeat analysis in a run utilizing pre-baked glassware where polypropylene tubes are noted to be used in the method. To ensure a minimal background of DBP, the glassware should be baked overnight at ≥ 200°C and cooled prior to use.

9.2. Samples which have suspect DEHP, DINP and/or DIDP values equal to or greater than the reporting limit, may be re-injected, along with the appropriate calibration set and quality control samples, using an optimized confirmation profile (non-routine).

9.3. The suspect test sample is confirmed to contain the specific analyte (s) if the following criteria are satisfied.

9.3.1. The retention time of the analyte (s) in the suspect sample match the retention time of the matrix-fortified standard run under the same experimental conditions to within ± 2.5%.

9.3.2. All the expected product ion transitions including the precursor ion listed in Section 7.3 must be present with measurable ion intensities.

9.3.3. The product ion intensity ratios in the suspect test sample agree, within limits given in NLO-FD003.00 Annex 1, to those of the matrix-fortified control sample fortified at or near the concentration of the suspect sample.

10. Test Reporting:

10.1. Results are reviewed by program supervisor before reporting or re-analysis of samples.

10.2. Method Characteristics [(1)]

Analytical Range - 1.0 – 20.0 µg/g, sample equivalents
Detection Limit - 0.5 µg/g, sample equivalents

Reporting Limit - 1.0 µg/g, sample equivalents
Tolerances[2] - 1.0 µg/g, sample equivalents

[1] The Measurement Uncertainty is to be re-calculated whenever a change that affects method accuracy, precision or sensitivity occurs. This information is to be prepared by the responsible program chemist and forwarded to the Section Head for review, with a copy provided through to the CVDR Quality Officer/Designate.

[2] Tolerance information is provided as guidance only; on-going consultation with the customer (CFIA-FSSD) is recommended to ensure the reporting limit continues to meet their needs. All results greater than the reporting limit, if confirmed, are reported.

11. Quality Assurance Plan:

11.1. Quantitative Determinative Performance Standards

Note: Jams, juices, jellies, powders, tea and syrup were validated in joint with the FDA laboratories (data on file, validation, August, 2011). Extension work to expand the commodity scope to include water, infant formula, baby cereals and processed baby foods was done in-house Jan-Feb 2012 (Report on file, June, 2012).

Correlation coefficient, R > 0.990
Reproducibility, %RSD (between labs) – not available
No false positives
No false negatives if > 0.5 µg/g, sample equivalents
% Accuracy, No systematic bias and no individual bias > 30%
Within run Repeatability, where n (# samples) =2, %RD ≤ 35%; n>2, %RSD=25
Between run repeatability, where n=2, %RD ≤ 50%; n>2, %RSD=35
% Recovery, MF/MM (MF/Chem), 100 ±20%.

List of abbreviations:

MF = Matrix-Fortified, extracted sample, spiked prior to extraction
MM = Matrix-Matched, extracted sample, spiked post extraction
Chem = Chemical Standard
%RD = % relative difference
%RSD = % relative standard deviation

Table of observed performance characteristics (August 2011 validation summary data).

	Matrix	BBP	DINP	DBP	DEHP	DNOP	DIDP
Within run Repeatability[1], %cv where n>2 *presented as the maximum observed	Cereal[1]	25	24	19	23	21	34[1]
	Infant formula, powder[1]	9	19	14	11	16	38[1]
	Infant formula, liquid *excludes one outlier	11	16	8	13	19	16
	Jarred Baby foods [1] (Fruit/Vegetable)	11	15	11	29[1]	8	10
	Bottled Water	2	3[3]	2	3	2	4

	Matrix	BBP	DINP	DBP	DEHP	DNOP	DIDP
	Powders[1]	16	90[1]	30[1]	52[1]	5	20
	Jam	16	13	20	8	7	20
	Jelly	7	10	25	23	17	7
	Juice[1]	18	51[1]	35[1]	15	50	18
	Syrup	9	7	26	15	5	6
	Tea[1]	4	35[1]	12	39[1]	7	8
Between run repeatability based on accumulated review of accuracy data, %cv, n>2	Cereal	26	32	22	43	30	27
	Infant formula, powder	24	45	35	27	35	27
	Infant formula, liquid[3]	11	16	14	15	20	15
	Jarred Baby foods (F/V)	11	17	23	24	12	15
	Bottled Water	14	21	24	8	19	41
	Powders	30	74	29	39	25	26
	Jam	10	10	13	12	7	36
	Jelly	11	6	13	11	23	11
	Juice	8	21	22	18	18	9
	Syrup	9	8	13	9	18	9
	Tea	15	28	27	39	28	27
% Recovery, average observed at validation MF/MM[3] (MF/Chem)[2]	Cereal	91 (56)[2.]	93 (86)	100 (96)	101 (99)	97 (85)	71 (74)
	Infant formula, powder	95 (83)	82 (83)	92 (80)	82 (87)	85 (84)	63 (60)[2.]
	Jarred Baby foods (F/V)	92 (103)	89 (91)	102 (104)	87 (81)	88 (85)	71 (157)[2.]
	Infant formula, liquid	112 (113)	108 (106)	130 (111)	120 (109)	106 (102)	113 (128)
	Bottled Water	86 (103)	57 (105)	92 (111)	86 (104)	82 (101)	94 (108)
	Powders[3, MF/MM data]	76 (85)	94 (103)	87 (83)	104 (98)	73 (88)	92 (88)
	Jam [3, MF/MM data (jam, jelly pooled)]	86 (87)	84 (95)	95 (99)	86 (90)	68[2] (101)	87 (75)[2]
	Jelly	(110)	(100)	(94)	(98)	(87)	(106)
	Juice[3, MF/MM data]	103 (99)	99 (89)	102 (97)	98 (94)	57[2] (91)	94 (96)

	Matrix	BBP	DINP	DBP	DEHP	DNOP	DIDP
	Syrup	(92)	(104)	(95)	(100)	(90)	(104)
	Tea	(86)	(90)	(85)	(112)	(85)	(86)

1. Where the within repeatability criteria within a specific matrix type exceeds 25% RSD (see validation summary below), standards addition approach to quantitation is recommended for that matrix type, for that specific analyte. Consult the program supervisor as required.
2. Where the observed % Recovery (MF/Chem) falls outside 100 ± 30%, matrix-based calibration standards are required for that matrix type, for that specific analyte.
3. Where noted, data updated with observed 2011-2012; 2012-2013 diagnostic performance information

11.2. Critical Control Points:

11.2.1. To avoid contamination from phthalates present in plastics, use glass labware wherever practicable. See Section 3, apparatus; Section 6, extraction.

11.3. Readiness to Perform (Training Plan)

Notes: As part of the analyst qualification process, an observation run is to be completed, whereby the analyst has the opportunity to work with an analyst experienced in the application of the procedure.

At the discretion of the program supervisor, phase I and II may be combined, for each run, setting up the calibration and system suitability standards required for phase I along with the required spikes for phase II.

11.3.1. Phase I: Phase I provides analyst(s) with the opportunity to demonstrate competency on instrument setup and evaluation of instrument system suitability data and is to include:

Two runs, setup and run on separate days to include a set of four chemical standards covering the analytical range of the method. The range might be set up to mimic the range used in a routine determinative run.

11.3.2. Phase II provides analyst with the opportunity to demonstrate competency on the analytical procedure, evaluation of results and reporting and is to include:

Two runs, done on separate days; each run to include a system suitability injections (chemical standard), a matrix-fortified calibration set and six analyst spikes, 3 levels in duplicate.

Submissions for review and approval to proceed to phase III include:
For each run, the worksheet/report (reviewed and approved by the supervisor); the supporting system suitability evaluation (analyst comments) as they relate to acceptance of instrument output, the run sequence, regression analysis(es), all chromatograms, as well as a summary of the analyst spike recoveries and precision.

All runs must be accounted for (including those which did not meet the test method acceptability criteria).

11.3.3. Phase III provides for an evaluation of the analyst's ability to obtain and produce an analytical result which is unknown to them and is to include:

Two quantitative determinative runs set up on separate days, each run to include the system suitability injections, a calibration curve, positive and negative quality control samples, the LOD spike and a minimum of 6 blind check samples.

Submissions for review and approval include:
For each run, the worksheet/report (reviewed and approved by the supervisor) which is to include the system suitability evaluation (analyst comments) as they relate to acceptance of instrument output, the run sequence, regression analysis(es), all chromatograms, as well as a quantitative presentation of the check sample results.

All runs must be accounted for (including those which did not meet the test method acceptability criteria).

11.3.4. The analyst qualification package is to include

For the phase I and II data, summaries of the analyst spike results (including an assessment of the recovery and precision data).

For the phase III data, a quantitative presentation of the check sample results.

All runs must be accounted for. The summaries are to be traceable to the run identifiers and traceable to the analytical instrument used at analysis.

11.3.5. Acceptability criteria. See 11.1, Performance Standards.

11.4. Intralaboratory Check Samples:

11.4.1. System, minimum requirements

Repeat samples - samples from previously analysed, one for every 20 samples analyzed, set up in the same run.

Positive Control Samples - at least one positive control is included in every run. The level is as defined within the text of the method.

Negative Control Samples - at least one negative control is included in every run.

Recovery Spikes – one matrix-matched spike is included per run. The analyte responses are evaluated against the comparable spike levels set up as matrix-fortified spikes (the calibration set).

11.4.2. Records are to be updated and reviewed for trends by the analyst with every run. Those records include:

All repeat sample results, one for every 20 samples analyzed as well as those repeated for review of quantitation.

Positive Quality results are recorded in a table and when sufficient data is available to generate control limits, the positive QC results are plotted into Individual, X and moving Range, R Statistical Process Control charts. Analyst qualification and validation data may be used to supplement charts.

Negative control results, qualitatively tracked as either detect or non-detect.

Recovery data is recorded in a table. This data may be used to prepare Individual, X and moving Range, R Statistical Process Control charts.

11.4.3. Acceptability criteria.

See Section 11.1, Performance standards.

Positive Control and Recovery Sample results, when plotted into an SPC chart, are to be reviewed for trends. SPC review criteria - results are within calculated control limits.

The negative control is generally ND for all analytes. A trace level of DBP is observed in the reagent and negative control extracts when extracts are prepared using the polypropylene tubes. The LOD spike response data are to be greater than reagent blank/negative control DBP response by at least a factor of 2.

When criteria are not met and/or trends observed, consult with the responsible supervisor. Investigate and identify probable cause, documenting this information with the run, noting actions taken on the necessary control charts/table.

11.5. Interlaboratory Check Sample: None available.

11.6. Uniform Analytical Standards:

11.6.1. When a new stock standard solution is prepared (Section 5) it is validated for use as per CVDR-S-0014, and is to include a review of the individual

preparation for possible cross contamination as well as a review of the concentration of the prepared stocks (prepared at the working standard concentration) relative to the current/old mixed working standard solution.

11.6.2. The concentration of the new stock standard solutions (section 5) is verified against the old stock solution using the mixed working standard, 1 µg/mL solution. Add 20 µL of the 1 µg/mL mixed Phthalate solution into 500 µL methanol. (40 ng/mL in-vial). Compare the results for the fresh preparation relative to the older preparation via the LC-MS/MS.

11.6.3. See 7.3 for suitability criteria of retention parameters.

11.6.4. See CVDR-S-0014 for repeatability criteria of responses.

11.6.5. If the repeatability criteria between the old and new preparations is exceeded, determine the source of the variance. The possible sources of variability include the instrument (review repeatability of injection), the dilution step done to prepare the working solution level and/or a possible weighing/transfer error when preparing the standard reference material for the stock solution. Keep these sources in mind when trying to determine why a stock solution is not validating as expected.

11.6.6. Records of the preparation details, the supporting chromatograms from the verification run(s), the calculation results (% difference of the retention times and the RPD data for response comparisons) and conclusions with regards to acceptance of the new standard preparations are kept in the Standards log book.

11.7. Sample Acceptability and Stability

11.7.1 Matrix of choice: Jams, Juices, Jellies, Teas, Syrups, Powders, water, infant formula (liquid, powder), infant cereal, jarred baby foods (fruit, vegetables)

11.7.2 Condition upon receipt: sealed container, not spoilt

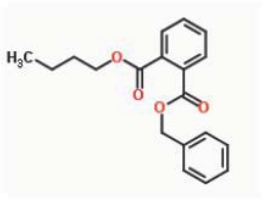
11.7.3 Sample storage: Unopened- store at room temperature unless stated otherwise on packaging. Refrigerate once opened and sub-sampled. Retain sample in original packaging whenever possible.

12 Revision Status:

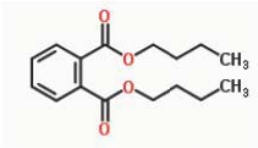
Note: Information provided below accounts for the changes made to warrant an update to this particular noted version.

Previous Version	Previous Version Revision Date	Paragraph revised, deleted, added	Reason for update	Original Author: Revision Author (if different)
NEW	NEW		Required in response to DEHP contamination of Taiwanese products (2011)	B. Shurmer/C. Neiser
Version 01	2011/12	Section 5, Section 10	Updated to include extension commodities; added CAS #'s for the reference standards; Added in two more deuterated standards (new)	C.Neiser
		Section 3,4, 5	Updated to include use of baked glassware, where appropriate. Removed use of pipettors (source of phthalate background too variable for routine use). Added scintillation vials.	
		Section 6	Provided detail for sample preparation specific to determinative runs. Added in an overnight freeze step of the extract to aid with de-fatting.	
		Section 7	LC and MS/MS parameters reviewed and updated. Minor edits to LC conditions; added in deuterated standard acquisition details; included additional (optional) transitions for confirmation; revised DIDP quantitation mass; provided system suitability detail	
		Section 8	Provided details for standards addition; provided details for approach to recovery calculations and quantitation options	
		Section 9	Provided details for DBP evaluation; noted non-routine MS/MS parameters may be used for confirmation purposes	
		Section 10	Provided further clarification on approach for standards addition and matrix based calibration needs; updated upper calibration range	
		Section 11	Removed reference to Quality Officer; updated records-keeping requirements and acceptance criteria; updated standards validation section (added reference to applicable SOP); updated list of suitable matrices and sample storage details	
		Section 12	Updated Revision table	
		Appendix	Chromatograms to be updated yet	

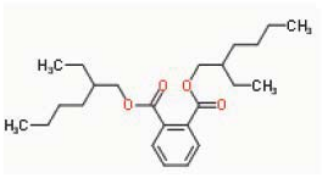
Appendix I - Structures



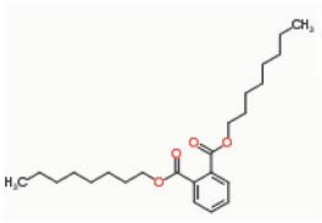
Benzyl butyl phthalate (BBP)



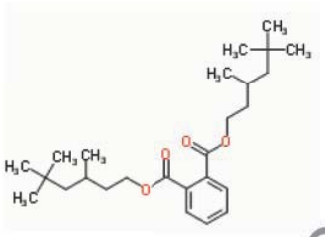
Dibutyl phthalate (DBP)



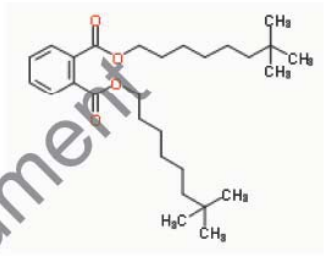
Di-ethyl-hexyl phthalate (DEHP)



Di-n-octyl phthalate (DINP)



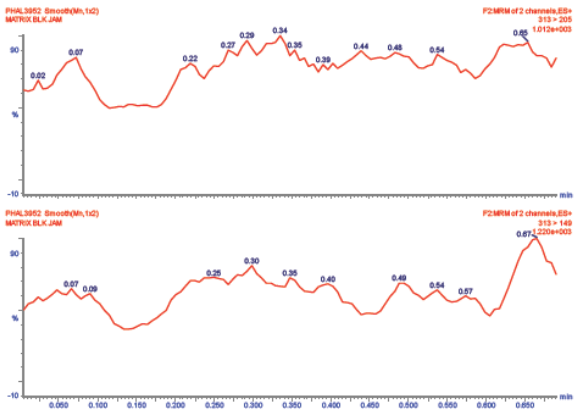
Di-iso-nonyl phthalate (DINP)



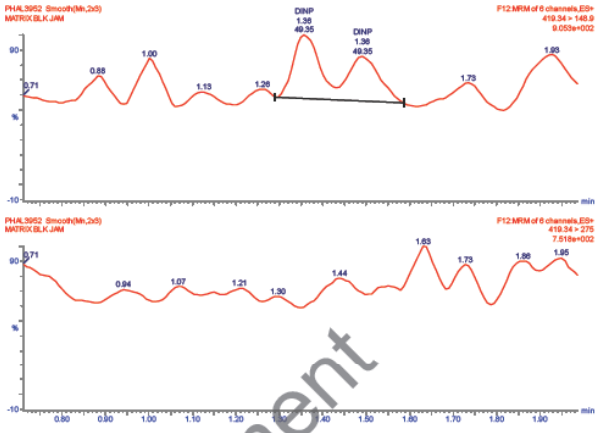
Di-iso-decyl phthalate (DIDP)

Appendix II - Typical Chromatograms, Extracted Jam, Blank

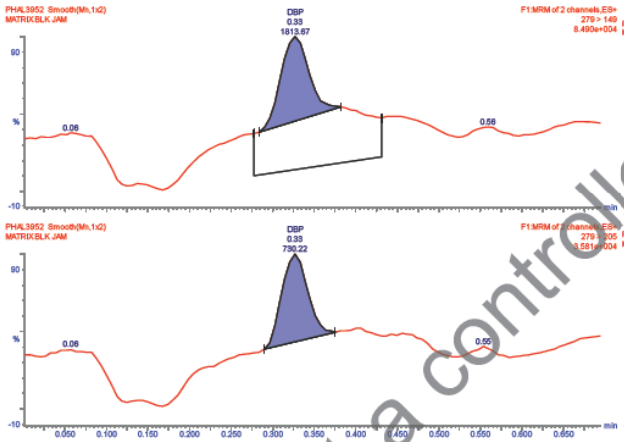
BBP



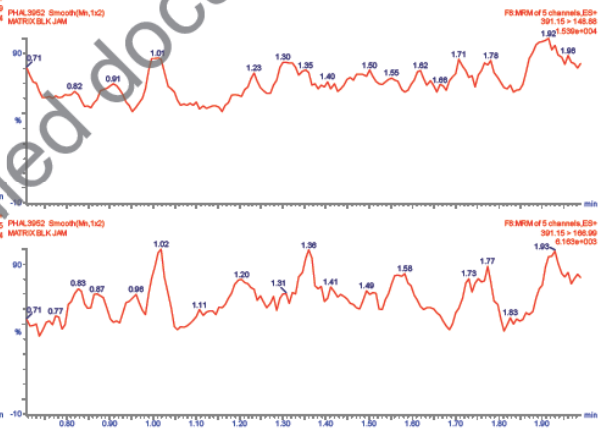
DINP



DBP



DEHP



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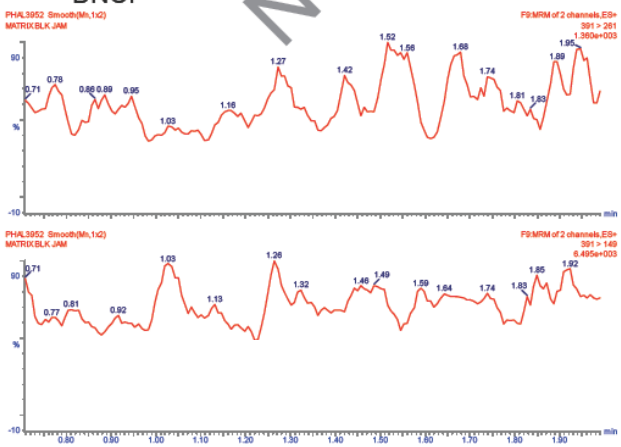

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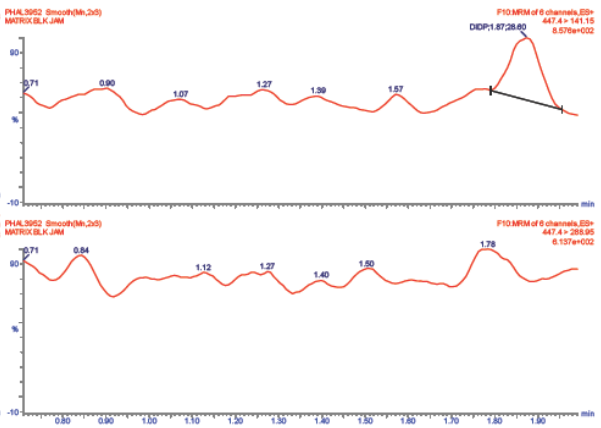
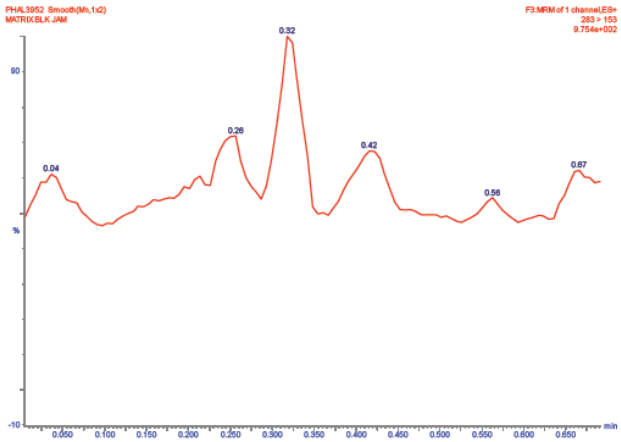
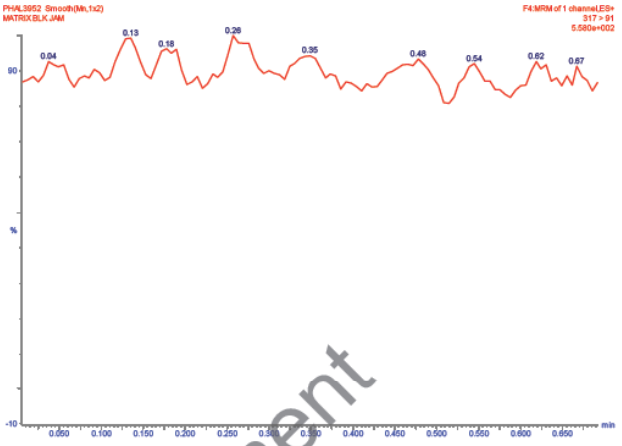


Appendix II - Typical Chromatograms, Extracted Jam, Blank, continued

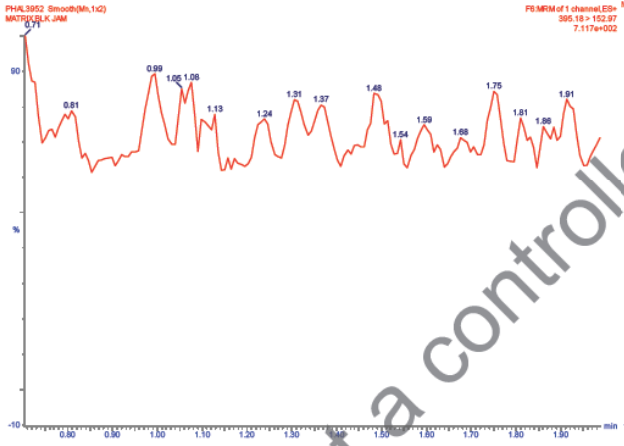
DBP-D4



BBP-D4



DEHP-D4



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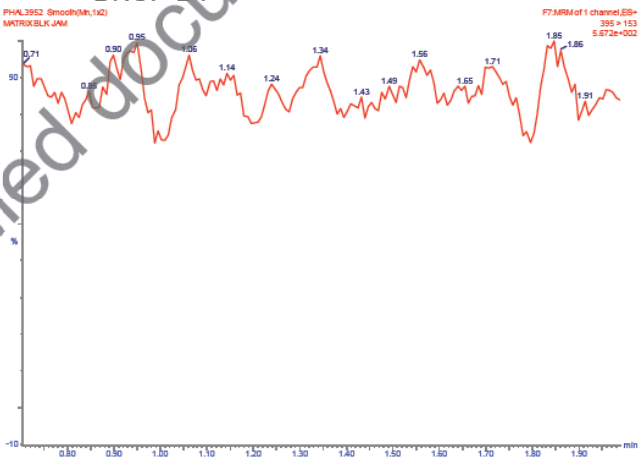

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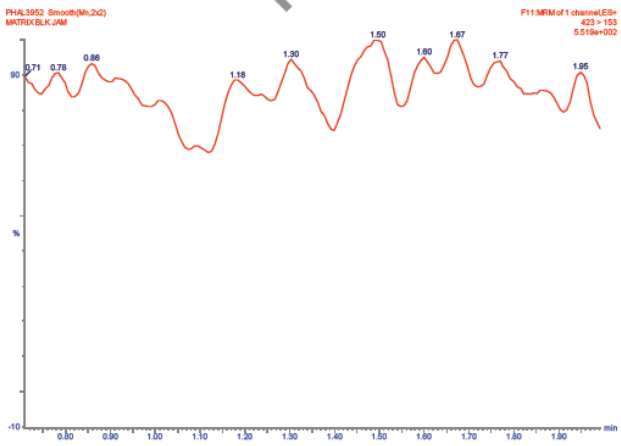
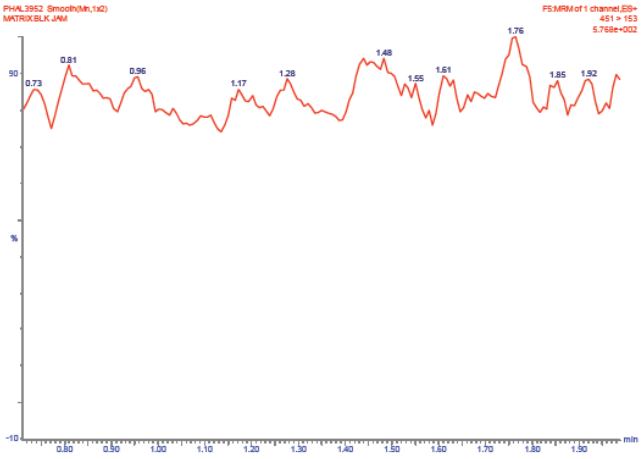
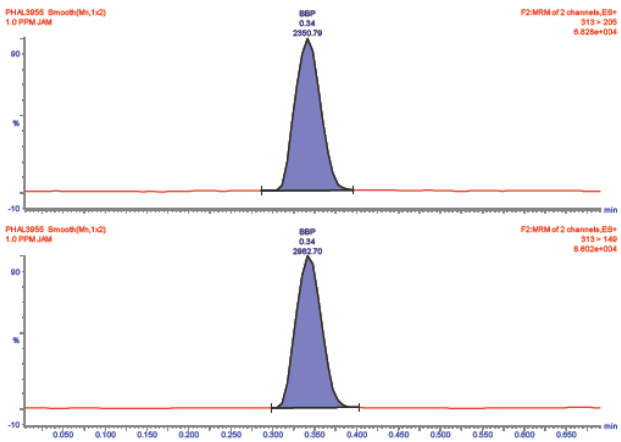


DIDP-D4

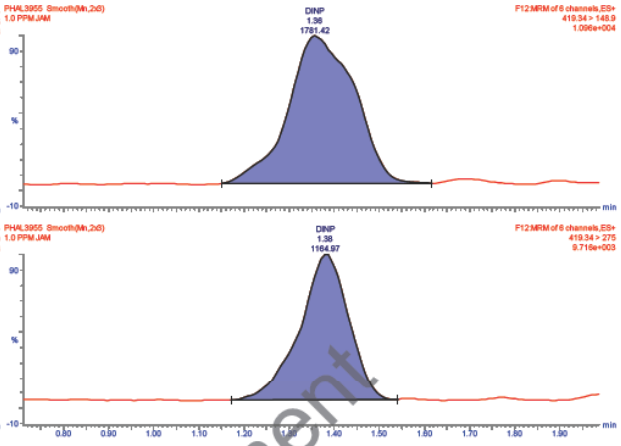


Appendix II - Typical Chromatograms: Extracted Jam, Fortified at 1.0 µg/g, Tissue Equivalents

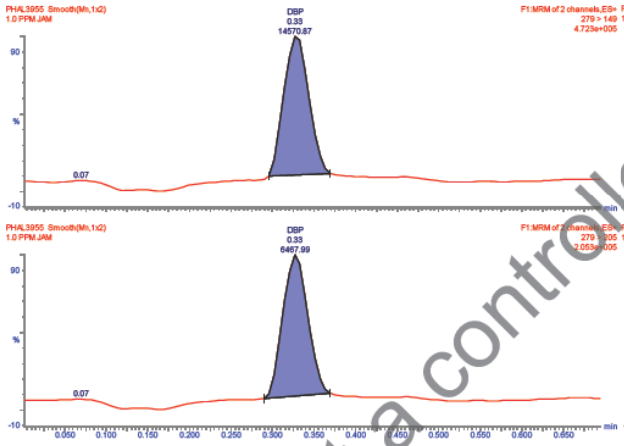
BBP



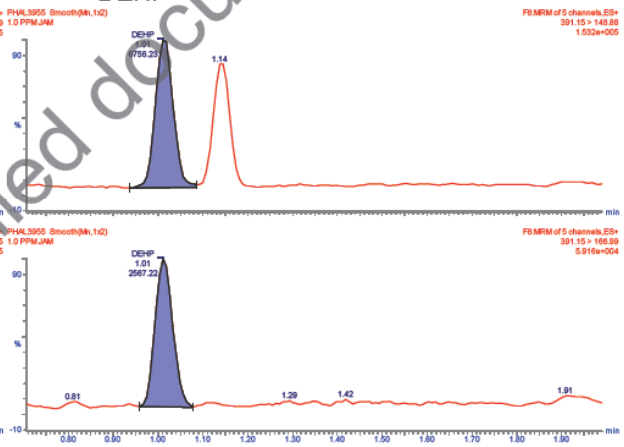
DINP



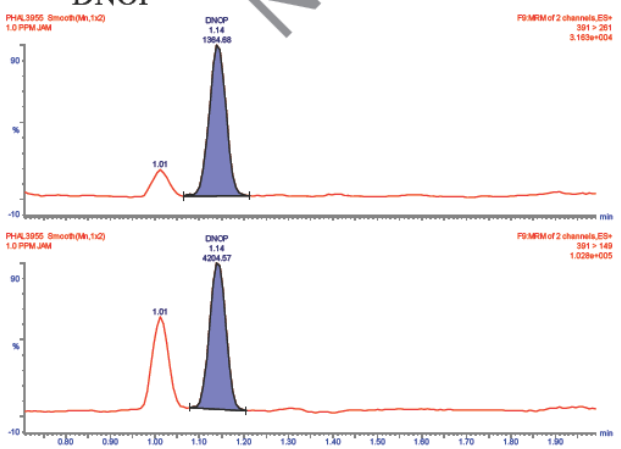
DBP



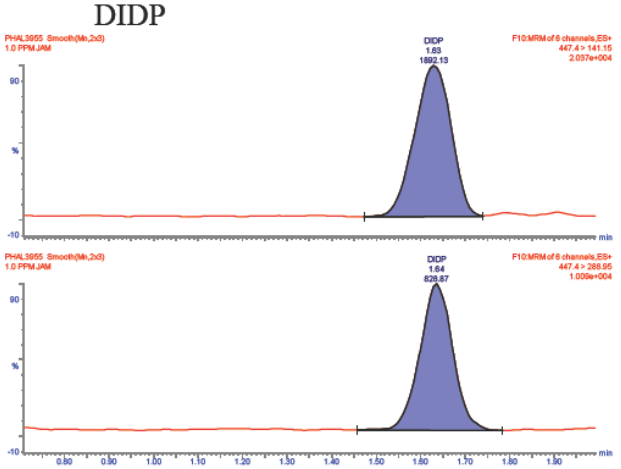
DEHP



DNOP

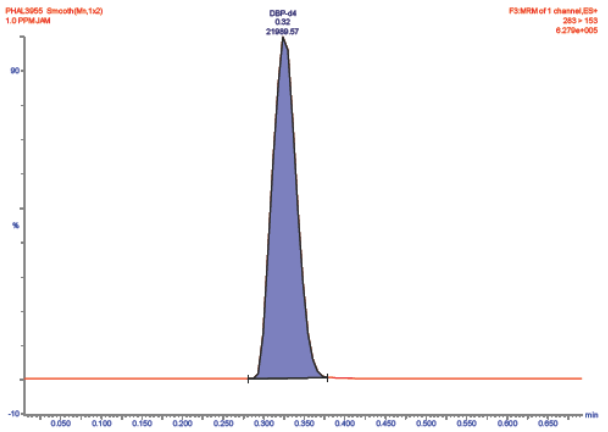


DIDP

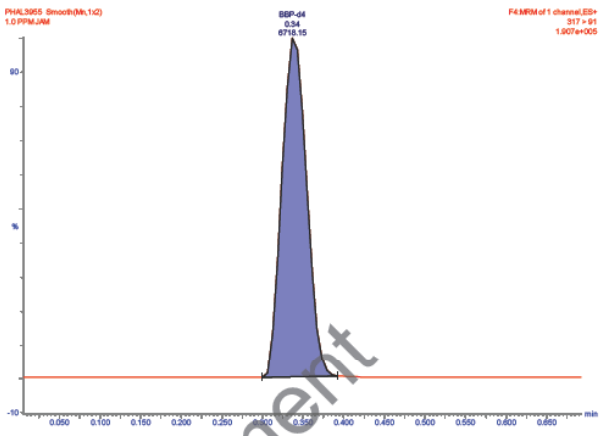


Appendix II - Typical Chromatograms: Extracted Jam, Fortified at 1.0 µg/g, Tissue Equivalents, continued

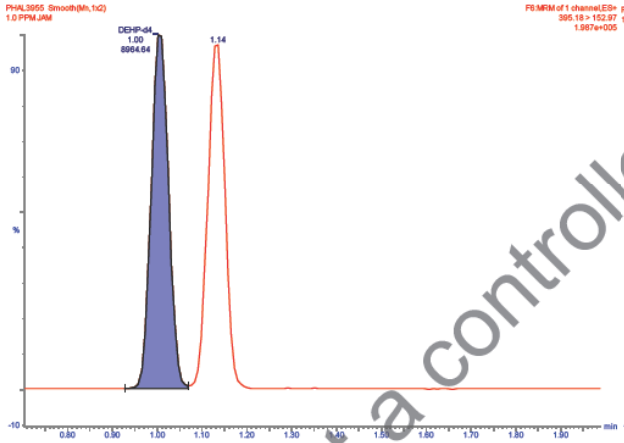
DBP-D4



BBP-D4



DEHP-D4



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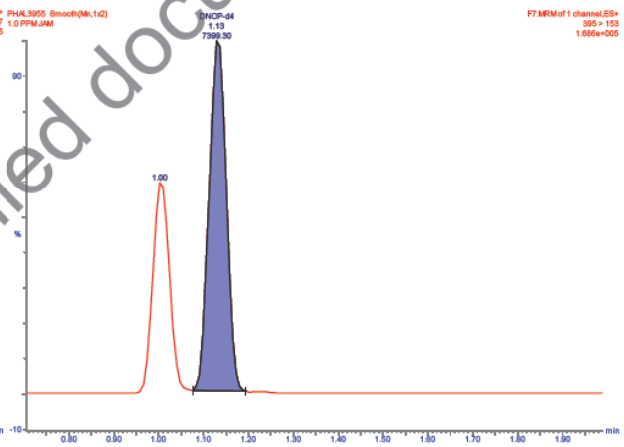

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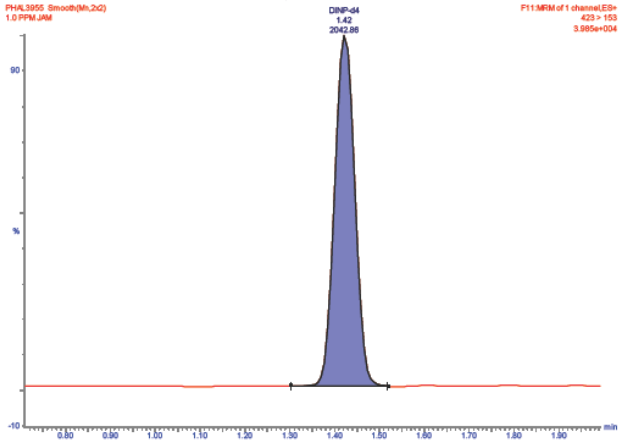
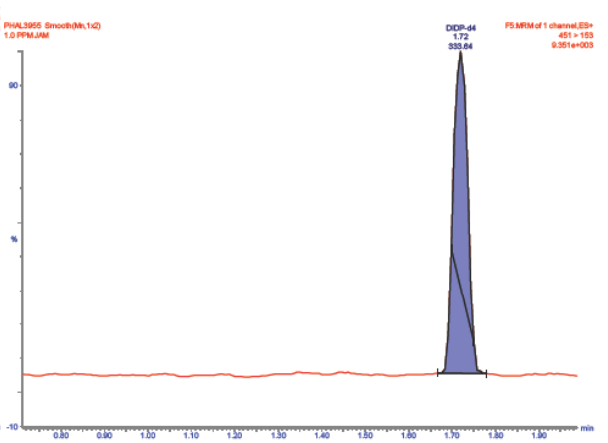


DIDP-D4



ANNEX B

BASIS OF PAYMENT

The Contractor will be paid in accordance with the following:.

1. <u>Contract period (01-05-2013 to 30-04-2016):</u>

For sample collection and analytical testing of allergens, chemical additives and residue contaminants described under article 6, 7, 11.2 and 11.3 and Tables 1 and 2 of the Statement of Work at Annex A:

At the following firm all-inclusive unit prices per Survey, inclusive of any costs associated with, but not limited to, sample collection, shipping and handling, analytical testing, deliverables, photos, reports including adhoc reports, and with confirmation procedures, as appropriate, as detailed below:

Total estimated Cost for sample collection and analytical testing : $_____

	01-05-2013 to 30-04-2014	**01-05-2014 to 30-04-2015**	**01-05-2015 to 30-04-2016**
Surveys	**Firm all-inclusive unit price per Survey**	**Firm all-inclusive unit price per Survey**	**Firm all-inclusive unit price per Survey**
Acrylamide in selected foods			Not applicable
Aflatoxins in selected foods			
Arsenic Speciation in selected foods			
Coumarin in selected foods			
Food colors in selected foods			
Fumonisins in selected foods			
Furans (including 2-methyl and 3-methyl furan) in heat-treated foods			
Glycoalkaloids in Potatoes			
Multi-Mycotoxin Analysis in selected foods			

PBDEs in selected foods			
Perchlorate in selected foods		Not applicable	
PFOS/PFOA in selected foods			
Phthalates in selected foods			
Undeclared multiple Allergens in pre-packaged foods			
Undeclared single Allergen in pre-packaged foods			

2. <u>For Optional period 1 (01-05-2016 to 30-04-2017):</u>

For sample collection and analytical testing of allergens, chemical additives and residue contaminants described under article 6, 7, 11.2 and 11.3 and Tables 1 and 2 of the Statement of Work at Annex A:

At the following firm all-inclusive unit prices per Survey, inclusive of any costs associated with, but not limited to, sample collection, shipping and handling, analytical testing, deliverables, photos, reports including adhoc reports, and with confirmation procedures, as appropriate, as detailed below:

Total estimated Cost for sample collections and analytical testing $_____

	Optional Period 1 (01-05-2016 to 30-04-2017)
Surveys	**Firm all-inclusive unit price per Survey**
Acrylamide in selected foods	Not applicable
Aflatoxins in selected foods	
Arsenic Speciation in selected foods	
Coumarin in selected foods	
Food colors in selected foods	
Fumonisins in selected foods	
Furans (including 2-methyl and 3-methyl furan) in heat-treated foods	
Glycoalkaloids in Potatoes	Not applicable
Multi-Mycotoxin Analysis in selected foods	
PBDEs in selected foods	
Perchlorate in selected foods	Not applicable

PFOS/PFOA in selected foods	
Phthalates in selected foods	
Undeclared multiple Allergens in pre-packaged foods	
Undeclared single Allergen in pre-packaged foods	

3. <u>For Optional period 2 (01-05-2017 to 30-04-2018)</u>

For sample collection and analytical testing of allergens, chemical additives and residue contaminants described under article 6, 7, 11.2 and 11.3 and Tables 1 and 2 of the Statement of Work at Annex A:

At the following firm all-inclusive unit prices per Survey, inclusive of any costs associated with, but not limited to, sample collection, shipping and handling, analytical testing, deliverables, photos, reports including adhoc reports, and with confirmation procedures, as appropriate, as detailed below:

Total estimated Cost for sample colllection and analytical testing $_____

	Optional Period 2 (01-05-2017 to 30-04-2018)
Surveys	**Firm all-inclusive unit price per Survey**
Acrylamide in selected foods	Not applicable
Aflatoxins in selected foods	
Arsenic Speciation in selected foods	
Coumarin in selected foods	
Food colors in selected foods	
Fumonisins in selected foods	
Furans (including 2-methyl and 3-methyl furan) in heat-treated foods	
Glycoalkaloids in Potatoes	Not applicable
Multi-Mycotoxin Analysis in selected foods	
PBDEs in selected foods	
Perchlorate in selected foods	Not applicable
PFOS/PFOA in selected foods	
Phthalates in selected foods	
Undeclared multiple Allergens in pre-packaged foods	
Undeclared single Allergen in pre-packaged foods	

Task Authorization Portion:

4. <u>For Optional Survey Packages:</u>

For Optional Survey Packages, on an "as and when requested" basis, during the Contract period and the Optional periods as described under articles 11.4.1 and 11.2 and 11.3 and Table 3 of the Statement of Work at Annex A:

At the following firm all-inclusive unit prices per Survey, inclusive of any costs associated with, but not limited to, sample collection, shipping and handling, analytical testing, deliverables, photos, reports including adhoc reports, and with confirmation procedures, as appropriate, as detailed below:

Total estimated Cost for Option Survey Packages: $_____

	Contract Period			Optional Period 1	Optional Period 2
	01-05-2013 to 30-04-2014	01-05-2014 to 30-04-2015	01-05-2015 to 30-04-2016	01-05-2016 to 30-04-2017	01-05-2017 to 30-04-2018
Survey Name	**Firm all-inclusive unit price per Survey**	**Firm all-inclusive unit price per Survey**	**Firm all-inclusive unit price per Survey**	**Firm all-inclusive unit price per Survey**	**Firm all-inclusive unit price per Survey**
Aflatoxins in selected foods					
Arsenic Speciation in selected foods					
Coumarin in selected foods					
Food colors in selected foods					
Fumonisins in selected foods					
Furans (including 2-methyl and 3-methyl furan) in heat-treated foods					
Multi-Mycotoxin Analysis in selected foods					
PBDEs in selected foods					
PFOS/PFOA in selected foods					
Phthalates in selected foods					
Undeclared multiple Allergens in pre-packaged foods					
Undeclared single Allergen in pre-packaged foods					

5. <u>For Expert Testimony services on an "as and when requested" basis, in accordance with 11.4.2 of the Statement of Work at Annex A:</u>

(a) Labour :

At firm all-inclusive daily rate as detailed below:

Total estimated Cost for Expert Testimony services $_____

Contract Period			Optional Period 1	Optional Period 2
01-05-2013 to 30-04-2014	01-05-2014 to 30-04-2015	01-05-2015 to 30-04-2016	01-05-2016 to 30-04-2017	01-05-2017 to 30-04-2018
Firm all-inclusive daily rate	Firm all-inclusive daily rate	Firm all-inclusive daily rate	Firm all-inclusive daily rate	Firm all-inclusive daily rate

Definition of a Day/Prorating: A day is defined as 7.5 hours exclusive of meal breaks. Payment will be for days actually worked with no provision for annual leave, statutory holidays and sick leave. Time worked (Days worked, in the formula below) which is less than a day will be prorated to reflect actual time worked in accordance with the following formula:

$$\text{Days worked} = \frac{\text{Hours worked}}{\text{7.5 hours per day}}$$

(b) **Travel and Living Expenses** : The Contractor will be reimbursed its travel and living expenses reasonably and properly incurred in the performance of the Work related to Expert Testimony, at cost, without any allowance for profit and /or administrative overhead , in accordance with meal, private vehicle and incidental allowances specified in Appendices B, C and D of the Directive http://www.njc-cnm.gc.ca/directive/travel-voyage/index-eng.php , and the other provisions of the Directive referring to "travellers", rather than those referring to "employees", are applicable.

All travel must have the prior authorization of the Technical Authority.

All payments are subject to government audit.

Total estimated Cost for Travel and Living expenses: $_____

6 . **For Additional Surveys services on an "as and when requested" basis, in accordance with articles 11.4.3, 11.2 and 11.3 of the Statement of Work at Annex A:**

At a firm all-inclusive hourly rate, inclusive of any costs associated with, but not limited to, sample collection, shipping and handling, analytical testing, deliverables, photos, reports including adhoc reports, and with confirmation procedures, as appropriate, as detailed below:

Total estimated Cost for Additional Surveys services$_____

	Contract Period			Optional Period 1	Optional Period 2
For additional survey(s)	01-05-2013 to 30-04-2014	01-05-2014 to 30-04-2015	01-05-2015 to 30-04-2016	01-05-2016 to 30-04-2017	01-05-2016 to 30-04-2017

	Firm all-inclusive hourly rate	Firm all-inclusive hourly rate	Firm all-inclusive hourly rate	Firm all-inclusive hourly rate	Firm all-inclusive hourly rate

7. <u>For Additional Analytical Testing Services on an "as and when requested" basis, in accordance with articles 11.4.4, 11.2 and 11.</u>3 of the Statement of Work at Annex A:

The firm all-inclusive unit prices per Survey specified below, when quoted by the Bidder, include any costs associated with, but not limited to, Sample Collection, shipping and handling, Analytical Testing, Deliverables, Photos, Reports including adhoc reports, and with Confirmation Procedures, as appropriate**.**

At a firm all-inclusive hourly rate, inclusive of any costs associated with, but not limited to, analytical testing, deliverables, photos, reports including adhoc reports, and with confirmation procedures, as appropriate, as detailed below:

Total estimated Cost for Additional Analytical Testing Services $_____

	Contract Period			Optional Period 1	Optional Period 2
For additional analytical testing services	**01-05-2013 to 30-04-2014**	**01-05-2014 to 30-04-2015**	**01-05-2015 to 30-04-2016**	**01-05-2016 to 30-04-2017**	**01-05-2016 to 30-04-2017**
	Firm all-inclusive hourly rate	**Firm all-inclusive hourly rate**	**Firm all-inclusive hourly rate**	**Firm all-inclusive hourly rate**	**Firm all-inclusive hourly rate**

Total estimated cost to a Limitation of Expenditure:_____
GST/HST extra, as applicable

ANNEX C

INSURANCE REQUIREMENTS

1.0 Commercial General Liability Insurance

1.1. The Contractor must obtain Commercial General Liability Insurance, and maintain it in force throughout the duration of the Contract, in an amount usual for a contract of this nature, but for not less than $2,000,000 per accident or occurrence and in the annual aggregate.

1.2. The Commercial General Liability policy must include the following:

(a) Additional Insured: Canada is added as an additional insured, but only with respect to liability arising out of the Contractor's performance of the Contract. The interest of Canada should read as follows: Canada, as represented by Public Works and Government Services Canada.

(b) Bodily Injury and Property Damage to third parties arising out of the operations of the Contractor.

(c) Products and Completed Operations: Coverage for bodily injury or property damage arising out of goods or products manufactured, sold, handled, or distributed by the Contractor and/or arising out of operations that have been completed by the Contractor.

(d) Personal Injury: While not limited to, the coverage must include Violation of Privacy, Libel and Slander, False Arrest, Detention or Imprisonment and Defamation of Character.

(e) Cross Liability/Separation of Insureds: Without increasing the limit of liability, the policy must protect all insured parties to the full extent of coverage provided. Further, the policy must apply to each Insured in the same manner and to the same extent as if a separate policy had been issued to each.

(f) Blanket Contractual Liability: The policy must, on a blanket basis or by specific reference to the Contract, extend to assumed liabilities with respect to contractual provisions.

(g) Employees and, if applicable, Volunteers must be included as Additional Insured.

(h) Employers' Liability (or confirmation that all employees are covered by Worker's compensation (WSIB) or similar program)

(i) Broad Form Property Damage including Completed Operations: Expands the Property Damage coverage to include certain losses that would otherwise be excluded by the standard care, custody or control exclusion found in a standard policy.

(j) Notice of Cancellation: The Insurer will endeavour to provide the Contracting Authority thirty (30) days written notice of policy cancellation.

(k) If the policy is written on a claims-made basis, coverage must be in place for a period of at least 12 months after the completion or termination of the Contract.

(l) Owners' or Contractors' Protective Liability: Covers the damages that the Contractor becomes legally obligated to pay arising out of the operations of a subcontractor.

(m) Litigation Rights: Pursuant to subsection 5(d) of the *Department of Justice Act*, S.C. 1993, c. J-2, s.1, if a suit is instituted for or against Canada which the Insurer would, but for this clause, have the right to pursue or defend on behalf of Canada as an Additional Named Insured under the insurance policy, the Insurer must promptly contact the Attorney General of Canada to agree on the legal strategies by sending a letter, by registered mail or by courier, with an acknowledgement of receipt.

For the province of Quebec, send to:

Director Business Law Directorate,
Quebec Regional Office (Ottawa),
Department of Justice,
284 Wellington Street, Room SAT-6042,
Ottawa, Ontario, K1A 0H8

For other provinces and territories, send to:

Senior General Counsel,
Civil Litigation Section,
Department of Justice
234 Wellington Street, East Tower
Ottawa, Ontario K1A 0H8

A copy of the letter must be sent to the Contracting Authority. Canada reserves the right to co-defend any action brought against Canada. All expenses incurred by Canada to co-defend such actions will be at Canada's expense. If Canada decides to co-defend any action brought against it, and Canada does not agree to a proposed settlement agreed to by the Contractor's insurer and the plaintiff(s) that would result in the settlement or dismissal of the action against Canada, then Canada will be responsible to the Contractor's insurer for any difference between the proposed settlement amount and the amount finally awarded or paid to the plaintiffs (inclusive of costs and interest) on behalf of Canada.

2. Errors and Omissions Liability Insurance

2.1. The Contractor must obtain Errors and Omissions Liability (a.k.a. Professional Liability) insurance, and maintain it in force throughout the duration of the Contract, in an amount usual for a contract of this nature but for not less than $1,000,000 per loss and in the annual aggregate, inclusive of defence costs.

2.2. If the policy is written on a claims-made basis, coverage must be in place for a period of at least 12 months after the completion or termination of the Contract.

2.3. The following endorsement must be included:

Notice of Cancellation: The Insurer will endeavour to provide the Contracting Authority thirty (30) days written notice of cancellation.

ANNEX D
TASK AUTHORIZATION FORM

PWGSC FILE NO.: _____ **CONTRACT SERIAL NO.:** _____

TASK NO.: _____ **AMENDMENT NO.:** _____

TITLE: _____

REASON FOR AMENDMENT, IF APPLICABLE:
_____
_____
_____

1. **DESCRIPTION OF THE WORK:** As follows _____ See attached _____

_____
_____
_____

Deliverables : As follows ____ See attached ____
_____
_____
_____

Delivery Date(s) :
_____
_____
_____

****

2. **COST BREAKDOWN**

A. **For Optional Survey Package:**

At firm all-inclusive unit price for *(insert Survey Package),* inclusive of any costs associated with, but not limited to, sample collection, shipping and handling, analytical testing, deliverables, photos, reports including adhoc reports, and with confirmation procedures, as appropriate, as detailed below:

Total estimated Cost for Option Survey Package $_____

B. **For Expert Testimony:**

(a) **Labour :**

At firm all-inclusive daily rate of $_____ for an estimated _____days.

Total estimated Cost for Expert Testimony: $_____

Definition of a Day/Prorating: A day is defined as 7.5 hours exclusive of meal breaks. Payment will be for days actually worked with no provision for annual leave, statutory holidays and sick leave. Time worked (Days worked, in the formula below) which is less than a day will be prorated to reflect actual time worked in accordance with the following formula:

Days worked = Hours worked
7.5 hours per day

(b) **Travel and Living Expenses** : at cost, without any allowance for profit and/or administrative overhead, in accordance with the meal, private vehicle and incidental expenses provided in Appendices B, C and D of the Treasury Board Travel Directive http://www.tbs-sct.gc.ca/hr-rh/gtla-vgcl/index_e.asp, and with the other provisions of the directive referring to "travellers", rather than those referring to "employees". All travel must have the prior authorization of the Technical Authority. All payments are subject to government audit.

Total estimated Cost for Travel and Living expenses: $_____

C. **For Additional Survey:**

At a firm all-inclusive hourly rate for (*insert Survey*), for an estimated __ hours.

Total estimated Cost for Additional Survey $_____

D. **For Additional Analytical Testing:**

At a firm all-inclusive hourly rate for _____ for an estimated __ hours.

Total estimated Cost for Additional Analytical Testing $_____

****

3. **BASIS OF PAYMENT:**

_____Limitation of Expenditure $_____ (GST/HST extra)

****

4. **METHOD OF PAYMENT:**

____Monthly payment

****

5. **APPROVALS:**

APPROVED: _____ _____ _____
Technical Authority Signature Date

APPROVED: _____ _____ _____
(if required) Finance/Administration (client) Signature Date

APPROVED: _____ _____ _____
(if required) PWGSC Contracting Authority Signature Date