FINAL REPORT

Monitoring of tropane alkaloids in foods

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1. Summary

A literature review was undertaken to understand the potential sources of tropane alkaloids (TAs) in the diet. Following the literature review a sampling plan was developed to carry out a survey of UK foods to determine their tropane alkaloid content. Sources of contamination were identified as *Datura* type seeds and the increase of *Convulvulus* type weeds in fields of food crops. Sampling was carried out for the UK FSA and as a part of a wider EFSA survey. A total of 286 samples were analysed for TAs and calystegines for the EFSA survey. 227 UK samples (197 cereal products, 10 green beans and stir fry vegetables and 20 teas) were analysed for TAs, and 59 UK samples (44 potatoes and 15 aubergines) were analysed for calystegines as part of the EFSA survey. An additional 52 UK retail samples (20 cereal based infant foods, 11 single grain flours and maize products, 12 oilseeds and 9 teas), and 17 single grain flours sampled directly at mills were analysed for TAs on behalf of the FSA.

Samples were analysed by LC-MS/MS, the method underwent validation and demonstrated satisfactory method performance by meeting the requirements of Commission Regulation (EC) No 401/2006 and SANCO/12751/2013 for method performance characteristics.

Overall the TA levels were low or not detected in most products, including, cereal based products (flours, maize products, breakfast cereals, bread, pasta, biscuits), oilseeds, and green & black tea. Atropine and scopolamine were the main TAs detected, but millet, sorghum and other flours contained the broadest range of tropane alkaloids, with one sample of yellow millet containing up to 10 different alkaloids. This sample contained 9.8 µg/kg atropine and 12.9 µg/kg scopolamine as well as 8 other alkaloids and had a total TA content of 33.6 µg/kg.

Cereal based infant foods are the only products with maximum levels of TAs of 1 µg/kg each for atropine and scopolamine (3). Four out of 66 UK samples were found to exceed these levels in this survey, (3/46 for the EFSA survey and 1/20 for the FSA survey). The highest level found was 3.73 µg/kg atropine, however up to 8 TAs were measured in some individual samples.

Sixteen out of 20 UK herbal teas contained detectable TAs. The highest level found was 129 µg/kg atropine in a peppermint tea. Tea infusions were prepared from a selection of 20 tea samples from the wider EFSA survey and analysed for TAs at RIKILT. On average, it was found there was a transfer efficiency of the alkaloids from the dry tea to the infusion of 47%.

Samples of potatoes and aubergines purchased in the UK were analysed for calystegines at RIKILT. Forty four samples of potatoes and 15 samples of aubergines were analysed. Calystegine A3, calystegine B2 and calystegine B4 were predominantly detected in potatoes. The highest level found was 310 mg/kg

calystegine A3. Calystegine A3, calystegine B1 and calystegine B2 were detected more frequently in aubergines. Calystegine B2 was detected at the highest concentration in aubergine, the highest level detected was 124.8 mg/kg.

2. Executive Summary

This report details the UK participation in a European wide survey for tropane alkaloids (TAs) in foods funded by EFSA and co-financed in the UK by the FSA.

The aims of the project were to carry out a literature review to understand the potential sources of tropane alkaloids in the diet, then to carry out a survey of foods from across Europe to determine their tropane alkaloid content. The literature survey was completed using published literature and and incidents reported by RASFF as information sources. The results of the literature review were used to develop the sampling plan. Sources of contamination were identified as *Datura* type seeds in some products and the increase of *Convulvulus* type weeds in fields of food crops.

Tropane alkaloids are molecules which contain a substituted tropane ring in their structure. They are secondary metabolites that occur naturally in many members of the plant family *Solanaceae* as well as several other genera such as *Datura*, *Atropa* and *Hyoscyamus*. Some tropane alkaloids have pharmacological properties and can act as anticholinergics or stimulants, the most well known being atropine and scopolamine. Some of these plants have been responsible for numerous episodes of human poisoning, either through deliberate misuse due to their hallucinogenic properties, or accidental ingestion, especially in children.

The samples identified as being the most important to sample were: cereal based foods including those that contain millet, sorghum and poppy seed; infant foods, including those containing millet, sorghum; some maize products; single grain flours, especially buckwheat; canned or frozen green beans and vegetable mixes and herbal teas. In total 286 UK samples were analysed for the EFSA survey for TAs and calystegines, (197 cereal products, 10 green beans and stir fry vegetables and 20 teas were analysed for TAs and 44 potato samples and 15 aubergine samples for calystegines). An additional 69 samples (52 UK retail samples comprising 20 cereal based infant foods containing millet, 11 single grain flours and maize products, 12 oilseeds and 9 teas), and 17 single grain flours sampled directly at mills were analysed for TAs.

Calystegines (another group of tropane alkaloids) were also included in the review. These are inherent toxins mainly found in *Solanaceae* plants. Potatoes and aubergines were included in the sampling plan as the major potential source of calystegines in the diet. A small number of peppers were also analysed by RIKILT.

A major conclusion of the review was that it is important to include as many TAs in the analytical method as possible, therefore the method included all TAs for which reliable analytical standards were available.

All samples were analysed by liquid chromatography coupled to tandem mass spectrometry. Method validation was carried out for cereals (single- flours and cereal based products) and teas. The method has been demonstrated to meet the

required performance criteria for atropine and scopolamine by the results of the validation exercise and QC data generated during the survey. In addition Fera participation in two Proficiency Tests run by the EURL for atropine and scopolamine in cereals and teas using this method gave 100% satisfactory results.

Overall the TA levels were low in most major staple (cereal based) foods. Single-grain flours, and most cereal products contained very low levels or no detectable TAs. Oilseeds and black and green tea also contained no significant levels.

The only maximum levels in force for the samples included in the survey are maximum levels of 1 µg/kg each for atropine and scopolamine in cereal based infant food as set out in Regulation (EU) 2016/239 (amending Regulation (EU) 1881/2006). Four infant food samples were found to exceed these levels, the highest level found was 3.73 µg/kg atropine. The samples were all analysed as received, but would require reconstitution before consumption.

Herbal teas were found to be commonly contaminated with TAs. Sixteen out of 20 teas from the UK contained detectable TAs. The highest level found in a UK herbal tea was 129 µg/kg atropine in a peppermint tea. Tea infusions were prepared from a selection of teas and analysed for TAs at RIKILT. On average, for the 20 tea samples tested from the wider EFSA survey, it was found there was a transfer efficiency of the alkaloids from the dry tea to the infusion of 47%.

UK purchased samples of potatoes and aubergines were analysed for calystegines at RIKILT. The two vegetables showed different contamination patterns. Levels in potatoes were on the whole higher than aubergines, with calystegine A3, calystegine B2 and calystegine B4 predominantly detected in potatoes. The highest level found was 310 mg/kg calystegine A3. Calystegine A3, calystegine B1 and calystegine B2 were detected in aubergines. Calystegine B2 was detected at the highest concentration in aubergine, the highest level measured was 124.8 mg/kg.

Overall levels of TAs found in the survey were low, with very few incidences of the atropine and scopolamine found. The relevance of the other compounds measured is hard to evaluate due to the lack of toxicity data about these compounds. Valuable additional information about the occurrence of TAs other than atropine and scopolamine has been obtained and so has added to the understanding of the possible presence of these compounds in food.

Herbal teas were the other product that contained the highest levels of TAs. This seems to be similar to the occurrence of pyrrolizidine alkaloids in teas, presumably caused by the unintentional inclusion of TA containing weeds in the tea product as green leaf plant teas such as peppermint were found to contain TAs at the highest levels.

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6. Glossary

EFSA	European Food Safety Authority
EU	European Union
EURL	European Reference Laboratory
Fera	Fera Science Ltd.
FSA	Food Standards Agency (UK)
HILIC	Hydrophobic Interaction Liquid Chromatography
LC-MS/MS	Liquid Chromatography tandem Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
MMRS	Matrix Matched Reference Sample
MRM	Multiple Reaction Monitoring
m/z	Mass to charge ratio
QC	Quality Control
RSD	Relative Standard Deviation
RSD_r	Repeatability
s : n	Signal to noise ratio
TA	Tropane Alkaloids
UPLC-MS/MS	Ultra Performance Liquid Chromatography tandem Mass Spectrometry

7. Introduction

Tropane alkaloids (TA) are secondary metabolites which naturally occur in plants of several families including *Brassicaceae*, and *Solanaceae* (e.g. mandrake, henbane, deadly nightshade, Jimson weed) (1). Although over 200 tropane alkaloids are known, (-)-hyoscyamine (an isomer of atropine) and (-)-scopolamine are the main alkaloids found in TA producing plants. TAs are antimuscarinic agents that are antagonists of the muscarinic acetylcholine receptors primarily present in the autonomic effector sites innervated by parasympathetic (cholinergic postganglionic) nerves but also in the central nervous system (CNS). In humans, the predominant peripheral antimuscarinic effects are decreased production of secretions from the salivary, bronchial, and sweat glands, dilation of the pupils (mydriasis) and paralysis of accommodation, change in heart rate, inhibition of micturition, reduction in gastrointestinal tone and inhibition of gastric acid secretion.

Chemical food contaminations leading to human intoxications are rare but do occur (2), but are only generally reported when they lead to patients being hospitalised. Often they have been caused by errors in preparing herbal preparations and tea. TAs can, furthermore, contaminate food when the weeds are harvested with the crop. Common practices for cleaning cereals are not always sufficient to remove the weed plant parts. The concentration of the tropane alkaloids thus entering the food chain is expected to be very low. More recently, it has been disclosed that a group of very polar tropane alkaloids, the calystegines are found in various edible plants of the *Solanum* genus and in the weed *Convolvulus*. This is a group of 14 different compounds with calystegine A3, calystegine B2 and calystegine B4 the most important (3). Expected levels in specific foods can be high.

In early 2016 Commission Regulation EU (No) 339/2016 came into force setting maximum limits of 1 µg/kg each for atropine and scopolamine in infant foods (3). In the European Food Safety Authority (EFSA) CONTAM Panel opinion on tropane alkaloids (4), only limited occurrence data were available. Most of the data used for the assessment was left censored, i.e. below limt of detection or limit of quantification, and a reliable exposure assessment could only be carried out for two tropane alkaloids ((-)-hyoscyamine (atropine) and (-)-scopolamine), for one food and one age class (toddlers). The CONTAM panel was unable to carry out a risk assessment due to the absence of useful occurrence data and therefore made the suggestion that additional data should be obtained and recommended occurrence data for various TAs as well as atropine and scopoloamine should also be collected. EFSA published a call for a European wide survey for TAs. RIKILT Wageningen UR, (The Netherlands), Investigación y Tecnología Alimentarias (IRTA, Barcelona, Spain), Vysoka skola chemicko-technologicka v Praze, (VSCHT, Czech Republic) and Fera Science Ltd., York, UK, formed a consortium that was awarded the contract to carry out the EFSA survey. The UK Food Standards Agency (FSA) provided matching funding for Fera to undertake the EFSA survey, as well as funding the analysis of additional samples collected in the UK. The additional UK sampling plan was designed in conjunction with the FSA with the aim of testing specific products of interest that were not included in the EFSA survey.

8. Aims and Objectives of the Study

A series of tasks were completed in order to achieve the following objectives on two Phases of the project.

8.1. Objectives and tasks for Phase 1

Identify relevant tropane alkaloids (TAs), in addition to atropine and scopolamine, that should be measured in the survey.

Identify at least five relevant food matrices that should be sampled. The feasibility of measuring these compounds by assessing analytical methods and the availability of standards should be described.

Prepare a method development and validation plan.

To achieve these objectives, a thorough review of available literature was carried out. The information from the literature review was used to inform and support the design of the sampling plans for the EFSA and FSA surveys.

8.2. Objectives and Tasks for Phase 2

Develop and validate methodology that would be suitable for the analysis of the sample types identified in the literature study for all the analytes of interest that had been identified. RIKILT, as EFSA lead partner, was responsible for co-ordinating this, and the supply of analytical standards and Quality Control (QC) materials to all partners.

Perform the sample collection for the EFSA study, and the FSA study agreed in accordance with the sampling plans agreed in Phase 1. Fera was required to purchase a target of 275 samples of cereals, cereal products, herbal teas and vegetable products to analyse for tropane alkaloids for the EFSA survey. It was agreed at an early stage that sampling would be carried out over two time periods; May-November 2015 and January-May 2016 to allow for possible seasonal variation to be taken into account. Fera was also required to collect a target of 50 UK specific samples, over the same two time periods.

Analyse the selected tropane alkaloids in the food samples collected using the validated analytical method. Samples were analysed using the methods provided by RIKILT and validated in-house at Fera using LC-MS/MS.

Compile the data from the analysis in the EFSA standard description format.

Prepare a final report for EFSA, that provides justification of the choice of analytes and sample types, the method used and its validation data, the sampling procedure and the results of all individual samples. RIKILT, as lead contractor produced an EFSA format report that was published on the EFSA website (5).

During sampling some additional samples were received, or the relative numbers in some categories were different. All samples received were included in the survey, therefore the total numbers analysed are slightly higher than the target numbers for both the EFSA and additional UK samples. In total 286 samples were analysed for EFSA, and 69 as additional UK samples. This report describes the Fera contribution to the overall project and the results of the additional UK samples that were analysed.

9. Literature review

9.1. Literature review parameters

To complete the first objectives stated above a full literature was carried out. The literature search was performed at the start of the project in March 2015, using search terms and an agreed strategy. Searches were carried out through the Fera Information Centre and its available on-line sources. The search did not include literature that was not in the English language. As well as published scientific literature the EU RASFF system was also searched for reports of incidents involving tropane alkaloids to help inform the sampling plan.

9.1.1. Results from the literature review

The literature study is presented in full in Annex 1 and is included as Annex A in the EFSA report EFSA-Q-2014-00320 (5). It reviews the occurrence of TAs in food plants and in co-occurring weed plants that both contain TAs and have a strong potential to contaminate foods. TAs for which no standard were believed to be available and cocaine and other TAs used for recreational drugs were excluded. The search provided about 3,500 results with the majority associated with atropine and scopolamine. In view of the considerable quantity of data concerning the occurrence and toxicity of atropine and scopolamine and the EFSA interest in the remaining TAs the focus of the review is on the less familiar TAs including calystegines. During the course of the project (until May 2016), new available information was added, where appropriate. No new extended literature search was performed after March 2015.

9.2. Conclusions and Recommendations from the literature review

Several conclusions and recommendations were reached from the literature review. The first was that information on the occurrence of TAs in food in the EU is scarce, in addition, the information available was not always pertinent to food, e.g. many reports related to horticulture or occurrence of TAs in non-edible plants or parts of plants such as roots and flowers.

9.2.1. TAs to be included in the survey

The majority of available information related to atropine and scopolamine and little information, (often dated), was available on the other TAs occurring in foods. Knowledge on toxic effects of TAs, other than atropine and scopolamine, was scarce, and therefore it has been concluded (e.g. EFSA opinion) that all TAs should be considered toxic and it is advisable to measure all TAs for which an analytical standard is available. A list of target compounds was prepared and is given in Table 1. These were the compounds for which reliable analytical standards could be obtained.

Table 1. Tropane alkaloids included in the survey

Tropane Alakloids included in LC-MS method	
Low molecular weight TAs	**Datura-type TAs**
6-Hydroxytropinone	O-Acetylscopolamine
Nortropinone	Anisodamine
Pseudotropine	Anisodine
Scopine	Apoatropine
Scopoline	Aposcopolamine
Tropine	Atropine
Tropinone	Homatropine
	2α-Hydroxymethyl atropine
Convolvulaceae-type TAs	Littorine
Convolamine	Noratropine
Convolidine	Norscopolamine
Convolvine	Phenylacetoxytropane
Fillalbine	Scopolamine

9.2.2. Potential contamination sources

The literature review gave extensive information on the occurrence of the different alkaloids in different plant species. Recorded cases of human intoxications with TAs were discussed, most of these incidents seem to have been caused by accidental ingestion of non-food plants or from mistaken identity of plants rather than food contamination incidents. Recent food contamination incidents from RASFF reports were also reported.

It is very difficult to predict which co-occurring weeds, and thus which TAs, will be present in the food in the EU. With respect to cereal crops contamination with weeds, the major weeds currently contaminating food crops in the EU are *Datura stramonium* and *Convolvulus arvensis*. The TAs tropine, tropinone, cuscohygrine, hygrine have been reported in *Convolvulus arvensis* and *C. sepium (Calystegia sepium*). Toxic weed plants from outside the EU likely to contaminate foods imported into the EU are Solanum species *S. ptycanthum*, *S. nigrum*, *S. viarum* and *S. torvum*. These plants are common in cultivated fields and have been harvested with peas, snap beans and soya beans. Other invasive solanums that are common in the USA and Asia are *S. viarum* and *S. torvum* (turkeyberry). Therefore there is a need to analyse all foods for all TAs for which an analytical standard is available.

9.2.3. Food types to be included in the sampling plan

The EFSA Opinion on tropane alkaloids in 2013 indicated a high proportion of cereal-based food for infants and young children were contaminated with TAs, cereal-based food intended for both toddlers and infants were also contaminated to a lesser extent (4). Contamination was also revealed in food and feed based on oilseeds, especially sunflower seeds, and in millet and related plants such as sorghum.

From RASFF reports, the greatest incidence of contamination has been caused by the presence of *Datura* seeds in millet (mainly organic and mainly originating in Austria) and *Datura* fruit (berries) in processed bean-based vegetables. Frozen green beans have also been contaminated with *Solanum* and poppy seeds with *Hyoscyamus* (henbane).

Although not wholly related to reports found on TAs during the literature review the widespread contamination of herbal tea products with pyrrolizidine alkaloids and a single incidence of contamination of tea prepared from marshmallow root with *A. belladonna* root suggested there might be a requirement to survey herbal teas from areas with potential tropane-containing plant growth.

Calystegines were also included in the review. Whilst there were many reports of contamination by *Datura* tropanes there were no reports of calystegine contamination of foods in which they do not occur naturally. There appeared to be widespread and sometimes heavy growth of *Convolvulus* in cereal fields, but it was concluded consumer exposure to calystegines would be greater and more sustained through consumption of potato and other vegetable products, e.g. aubergine.

9.3. Recommendations from the Literature review

9.3.1. Recommendations - Foods to be analysed for co-occurring TAs, (e.g. atropine, scopolamine, tropine, convolvine etc.)

- Processed cereal foods intended for babies, infants and toddlers (include a high proportion of organic products).
- Processed and unprocessed cereal foods for adults, including some samples with poppy seeds and products containing millet (include a high proportion of organic products).
- Processed foods and flours based on buckwheat, including some samples with poppy seeds products containing millet (include a high proportion of organic products).
- Canned and frozen vegetables with a high proportion of green beans.
- Tea, specifically herbal teas (based on recent experience with pyrrolizidine alkaloids).

9.3.2. Recommendations - Foods recommended to be analysed in the EFSA survey for inherent TAs (calystegines)

- Potato products of all types and cultivars to be analysed as priority crop.
- Aubergines - to be analysed based on consumption patterns in the EU.

In addition it was also recommended:

- To include samples from more than one harvest year.
- Food samples should be obtained from as many different sources as possible, including from outside the EU.

10. Sampling

10.1. Sampling Plan

Following the literature review the sampling plan was developed.

Sampling for UK samples was conducted by Fera. Samples were purchased throughout the UK and prepared according to a protocol agreed with EFSA and the FSA.

Sampling was conducted during the time periods May to December 2015 and January to May 2016. Samples were purchased from retail outlets around York, and some were purchased in Scotland. A significant proportion were also purchased from specialist suppliers and health food companies via the internet, these were from a variety of UK locations. As far as possible the sampling scheme from Commission Regulation (EC) No 401/2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs was followed. A minimum of 3 packs of the same batch were purchased and combined to produce a sample of a minimum 1 kg. It was possible to achieve this when Fera staff made the purchases in store, however the on-line retailers were not always able to fulfil this, e.g. they did not supply sufficient number of packs, or supplied packs from different batches and therefore some samples were less than 1 kg.

Samples were stored under suitable dry conditions, frozen if necessary, before being prepared and homogenised.

10.2. Sampling Plan and Samples – EFSA samples

The final sampling plan that was used for purchase of the samples is given in Table 2. It was agreed that the tea samples would include; chamomile, mint, rooibos (redbush), fennel, lemon balm, and nettle. Food products from organic production were collected for all food categories, approximately 25% of the samples were organic.

Table 2. Sampling Plan for EFSA survey samples.

Food category	**May/Dec 2015**	**Jan/May 2016**
	Total	**Total**
Single grain flours available at retail stores	**22**	**19**
Buckwheat	11	10
Millet/Sorghum	11	9
Cereals available at retail stores	**74**	**71**
Cereal based food for young children (age 6- 36 months)	20	10
Biscuits, cake other cereal based foods for young children (age 6-36 months)		15
Breakfast cereals	18	17
Bread/pasta	18	3
Biscuits, cake other cereal based foods	18	22
Maize products		4
Other products - retail	**0**	**30**
Herbal teas	0	20
Legumes, frozen beans and stir-fry mixes	0	10
Food plants from the Solanaceae family	**31**	**29**
Potato	23	22
Aubergine	8	7
Total	**127**	**149**

10.3. Sampling Plan and Samples – Additional UK samples

A sampling plan was agreed with the FSA for the additional target of 50 UK samples. The FSA specifically asked for samples of oilseeds, infant foods that contained buckwheat, millet or sorghum as an ingredient as well as products of EU origin and ethnic origin (i.e. Asia, Africa) to be collected as these were also identified as a potential source of tropane alkaloids but were not included in the EFSA sampling plan. The plan included green and black tea samples as these had been excluded from the EFSA survey. In addition to the samples collected above, nabim (National Association of British and Irish Millers) supplied 17 samples of mainly UK grown and milled cereals sampled directly at UK mills. The final sampling plan for additional UK samples is given in Table 3.

Table 3. Sampling Plan for additional UK samples

Food category	**FSA**			
	Round 1		**Round 2**	
	EU	**ethnic**	**EU**	**ethnic**
Infant foods containing buckwheat, sorghum or millet	7	3	7	3
Polenta and similar maize products	4	1	0	1
Single grain flour (retail)	3	2	0	0
Single grain flour (mills)	17			
Other products available at retail stores				
Oilseeds – linseed/flax	3	0	2	0
Oilseeds - others	1	2	0	2
Green tea	0	0	4	
Black tea	0	0	5	
Total	***35***	***8***	***18***	***6***
	Total	43	Total	24

10.4. Sample Homogenisation and Preparation for Analysis

Sample preparation was carried out according to a protocol that was agreed between the project partners and EFSA at the start of the project and complied with the requirements of Regulation (EC) (No) 401/2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. In all cases where multiple packs were purchased these were combined to produce a composite of ~1kg. The whole sample was milled and homogenised to ensure sample homogeneity. Cereals and tea samples were milled to a fine powder using a Retsch centrifugal mill.

All vegetables, including potatoes and aubergines were cryogenically milled. Samples of potatoes and aubergines bought in the UK, were cryomilled at Fera and then sent frozen by courier to RIKILT for calystegine analysis. In all cases samples were subdivided into three aliquots and were stored at -18°C after preparation until analysis.

11. Materials and Methods

11.1. Chemicals and Reagents

For extraction, methanol (HPLC grade) (Sigma-Aldrich, Gillingham, UK) and water (18.2 MΩ/cm Purelab Ultra laboratory purification system) (Elga, Marlow, UK) and formic acid solution (Fisher) were used. 7 N ammonia in methanol used for elution of solid phase extraction (SPE) cartridges was from Sigma-Aldrich. Strong Anion exchange cartridges - Strata-X-C 200 mg/6 ml, 33 µm (Phenomenex) were used to clean-up sample extracts. Methanol, acetonitrile, (UPLC/MS grade) (Biosolve, Dieuze, France via Greyhound, Birkenhead, UK) and ammonium carbonate were used for eluent preparation for LC-MS/MS analysis.

All calibration standards were sourced and verified by RIKILT using LC-MS and in some cases NMR. RIKILT supplied all analytical standards to the project partners to help ensure parity and consistency in results between the partners. To compensate for matrix effects during LC-MS/MS analysis, isotopically labelled internal standards, atropine-d3 and scopolamine-d3, were used. No other isotopically labelled standards were available. A list of all the TAs included in the method is given in Table 1.

11.2. Method Development and Validation

11.2.1. Validation Analyses

The methods used were developed and in-house validated by RIKILT and copies of the Standard Operating Procedures (SOPs) were distributed to all partners in the EFSA project. The final method used by all partners for TAs is given in Annex 2. RIKILT sourced and provided all analytical standards and QC materials and distributed these to the partners. Each partner undertook in-house method validation for the methods supplied by RIKILT for cereal products and teas. It was agreed that as only a small number of green bean / vegetable mix samples were included that validation was not required and an overspike of each sample would be included in that analytical batch.

Validation for calystegine analysis was not done at Fera as RIKILT analysed the UK samples for calystegines. The samples were prepared at Fera and analysed by RIKILT. The method was validated by the three partner laboratories, and the SOP of the method used is given in Annex 5.

11.2.2. Validation Criteria

There are no specific regulations or guidance documents that stipulate how to validate a method for the quantification of plant toxins in food products. Therefore it was decided, that the guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed SANCO/12751/2013 (6) would be used. Regulation (EU) 2016/239, that introduced limits for tropane alkaloids in infant foods, makes no specific reference to method performance characteristics. However, it does state that sampling for control of plant toxins should be performed in accordance with the rules of Regulation (EC) 401/2006, this regulation also sets method performance criteria for mycotoxins analysis.

It was decided to apply the general requirements on performance characteristics derived from SANCO/12751/2013 and Regulation (EU) No 401/2006 (amended by Regulation (EU) No 519/2014) for mycotoxins for the validation and analysis of tropane alkaloids.

The performance parameters are given below:

LOD: 3 times S/N calculated from lowest matrix or extract spiked sample

LOQ: 10 times S/N calculated from lowest matrix or extract spiked sample

Linearity: ≥0.99 for matrix or extract matched calibration curve

Recovery: 60 to 120 % for *Datura*-type TA; 70 to 110 % for calystegines

Repeatability (RSDr): ≤20 %

Reproducibility (RSDR): 2 × value derived from Horwitz Equation (modified by Thompson) for Datura-type TA; 2 × value derived from Horwitz Equation for calystegines

Measurement Uncertainty (U): <50 %

The linearity of the LC-MS/MS measurement was established through calibration standards in solvent, covering the relevant concentration range. From these initial in-house validations, the linearity, recovery, repeatability, selectivity, LOQ and limit of detection (LOD) were derived. In addition, the stability of retention time and ion ratios in solvent standards and extracts were determined.

Identification of analytes was based on retention time and ion ratio of coinciding peaks for at least two diagnostic transitions in the correct abundance ratio. The LC-MS SOP states that Retention Time should be within 2.5 % of the mean of the standards. The ion ratio of the two diagnostic ions (least abundant/most abundant) in the samples should be consistent with that obtained during validation and not deviate more than ± 20 %.

11.3. Validation plan

11.3.1. Validation plan for TAs in cereal products

A validation protocol for cereals was produced by RIKILT and all partners followed this. A summary is given in Table 4. The validation involved the analysis of replicate samples at different spiking concentrations (1, 5 and 25 µg/kg). Each batch contained one main product that was spiked at three levels, as well as other, similar matrices, that were included as Quality Control (QC) samples. The validation series covered the following products: breakfast cereals, single grain flours, bread, and cookies. Full validation (3 series) was carried out for breakfast cereals, 1 validation series was performed for each of the other matrices (Table 4).

Table 4. Samples selected for validation.

Matrix	Product 1	Product 2	Product 3
Breakfast cereals	Multi-grain based cereal for young children	Wheat bran flakes for breakfast	Muesli with fruit and nuts
Single grain flours	Buckwheat flour	Millet flour*	Sorghum flour*
Bread	Wheat bread	Multi-grain bread with seeds*	White bread*
Cookies	Biscuits with fruit	Sweet plain biscuits*	Biscuits for children*

- Products listed with an * were included as additional QC samples in the validation of the method (when validating product 1). For breakfast cereal samples product 2 and 3 were used as QCs when validating product 1; product 1 and 3 were used as QC for product 2 validation, and products 1 and 2 were QC for product 3 validation.

11.3.2. Validation plan for herbal teas

A second protocol for validation of the method for teas was also received from RIKILT, which followed the same principle. This used green tea as matrix to be validated, as it was difficult to find a blank tea matrix of any of the tea types that were included in the survey. The validation plan was followed with some additional analyses for black tea and all the product types selected for the survey.

11.3.3. Validation for calystegines

For potato and aubergine no naturally blank materials were available. Therefore it was decided to use naturally incurred materials and check the method for repeatability and extraction efficiency by means of these materials. The extraction efficiency was determined by extracting the samples twice.

11.4. Analytical Method - Extraction and Clean-Up

The Standard Operating Procedure (method description, SOP) for extraction, clean-up and analysis of tropane alkaloids was provided to all partners by RIKILT. The final version used for the survey and method validation is included here as Annex 2. Samples were extracted by shaking with an acidified methanol and water mixture. After centrifugation an aliquot of extract was cleaned up by strong cation exchange SPE. The cleaned-up extracts were evaporated to dryness, reconstituted and filtered before being analysed by LC-MS/MS.

As LC-MS/MS was used the accredited SOP for LC-MS analysis at Fera was followed. This stipulates criteria for ion ratio and retention time acceptance following the criteria outlined in SANCO/12571/2013 (Pesticides criteria document).

Further details of the LC-MS/MS conditions methods used at Fera are given below in section.

11.5. Preparation and extraction of herbal tea infusions

(Herbal) tea infusions were prepared at RIKILT according to a standardised protocol ISO 3103 (ISO, 1980) (8). Sample portions of 2 gram of dry, ground, homogenised (herbal) tea were transferred to paper tea filter bags (t-sac size 2, t-sac, Hannover, Germany), which was positioned in a 250 ml beaker. Boiling water (150 ml) was poured into the beaker, making sure that the tea bag was fully immersed. The sample was left to stand for 4.5 min, and subsequently, the tea bag was gently swirled around by hand in the beaker for 30 s. The tea bag was removed from the beaker and before disposal most of the adhering infusion was gently pressed out into the beaker. An aliquot of 37.5 mL tea infusion was transferred to a polypropylene tube of 50 mL and 75 µL of formic acid was added. The tube was centrifuged for 15 min at 3500 rpm and all of the supernatant was used for SPE clean-up.

For clean-up by SPE, an OASIS MCX 150 mg/6 cc (Waters, Milford, MA, USA) was used. The cartridges were conditioned with 6 mL of methanol and equilibrated with 6 mL 1% formic acid in water. The cartridges were loaded with 37.5 mL of tea infusion, washed with 6 mL methanol/water/formic acid, 75/25/1 (v/v/v) and dried under vacuum (using a vacuum manifold) for 5-10 min. TAs were eluted from the cartridges with 6 mL of methanol containing 0.5% ammonia (dry or added from 25% conc. ammonia solution). Further steps in the sample preparation were identical as described above for cereal-based products and dry (herbal) teas.

11.6. LC-MS/MS analysis for tropane alkaloids in cereals and teas

11.6.1. LC-MS/MS Instrument settings

For all LC-MS/MS methods the following conditions and equipment were used:

Chromatograph:	Waters Acquity UPLC
Autosampler:	Waters Acquity UPLC
Ionisation mode:	Electrospray positive
Capillary voltage:	1 kV
Desolvation temperature:	500 ^{o}C
Desolvation gas flow rate:	1000 L/h
Nebuliser gas flow:	7 bar
Source temperature:	150 ^{o}C
Cone gas flow rate:	100 L/h
Collision gas flow rate:	0.15 mL/min (3.5 mbar)
Resolution:	Unit mass
MRM conditions:	As SOP (Annex 2 section 7.2.3)

The SOP provided suggested LC-MS/MS parameters, but with all methods of this type some optimisation for each specific instrument is required to ensure the best performance of the method. Therefore the source tune parameters above differ slightly from those in the SOP (Annex 2 section 8.2.1), as these were found to give the best results for the instruments used for the study.

11.6.2. LC-MS/MS Method – Chromatography conditions

The following chromatography conditions were used:

Column:	Acquity BEH C_{18} 1.7 µm (150 x 2.1 mm) (Waters)
Column temperature:	50 ^{o}C
Injection volume:	2 µL
Mobile phase A:	10 mM ammonium carbonate in water
Mobile phase B:	Acetonitrile

The LC gradient profile for the determination of tropane alkaloids is given in Table 5. The gradient profile differs slightly from that in the SOP (Annex 2 section 8.2.2) by the addition of the flush step at 99 % B. This was introduced to ensure the column and LC system were clean after each injection and prevent late eluting material carrying over from one injection to another.

Table 5. Gradient timetable:

Time / min	% B	Flow Rate
0.0	0	0.40
2.0	0	0.40
12.0	60	0.40
12.2	99	0.55
15.0	99	0.55
16.0	0	0.55
19.0	0	0.55
19.1	0	0.40
20.0	0	0.40

11.7. Data Analysis and Quantification

MassLynx software (Waters) was used for data-evaluation. Peak assignment and integration were manually verified by the operator. Quantification was based on multi-level calibration using solvent standards. Where available a ^{3}d-labelled internal standard was used to internally standardise the method, and inherently correct analytical results.

11.8. Quality Control

All survey samples analysed at Fera were analysed with an accompanying overspiked sample so specific recovery information was obtained for each sample. Any sample found to be positive was repeated, with appropriate dilutions, to confirm the residues and levels present. In some cases where there were large concentration differences in the analytes in the samples this meant the sample was reanalysed multiple times at different dilution rates.

For each batch of survey samples, one or more spiked samples (QC materials supplied by RIKILT) were included to assess method performance between batches and recovery for different commodities. MMRS samples (‘after-spikes’) were also included to allow an assessment of matrix effects and ‘true’ recovery. All calibration standards were supplied by RIKILT so all partners used common calibrants. Blank samples and spikes were supplied by RIKILT and were included in each batch. Solvent based calibration solutions were used for analysis and the QC in each batch increased instead of using matrix matched calibrants.

During the survey the European Reference Laboratory (EURL) for Mycotoxins organised two proficiency tests (PTs) for the analysis of atropine and scopolamine. One was for cereal products and the other was for tea samples. Analysis of these PT samples was carried out at the same time as the survey analyses and results were submitted to the EURL. Fera performed well in both PTs, obtaining satisfactory Z-scores for all results for atropine and scopolamine for all matrices. This demonstrates the method is working well for atropine and scopalamine.

11.9. Reporting of Results

The limit of detection (LOD) of the method was defined as the concentration of each analyte required to give a chromatographic peak with signal to noise (s:n) ratio ≥ 3. This was assessed by extrapolation from the s:n ratio of each analyte in the lowest calibration standard and then applying a correction for recovery to determine the LOD in matrix. The limit of quantification (LOQ) of the method was defined as the concentration of the lowest calibration standard with s:n ≥ 3 and with a peak area fitting a calibration line with residuals ≤ 30 % and r2 ≥ 0.95, again with a recovery correction applied to obtain the LOQ in matrix. Results were expressed as a numerical value corrected for recovery where the residue was above the LOQ. Where no residue was detected the result was reported as <LOD, and the calculated (corrected) LOD given.

Results were assessed and the recovery value calculated for individual analytes in all samples. Each sample was assessed and an individual limit of quantification (LOQ) calculated. This took into account the apparent recovery from a spiked sample, therefore samples with a lower recovery had a higher apparent LOQ than might be suggested by measuring signal to noise (s : n) from solvent calibration standards. This is a 'truer' representation of what could be measured in each sample type. Results are reported corrected for recovery and are in µg/kg, apart from calystegine results which are in mg/kg. Where labelled internal standard was added to the sample before extraction recovery correction was inherent to the procedure. For final reporting to EFSA a standard approach was adopted by RIKILT where the lowest calibration standard, or that which gives acceptable signal:noise was used as the reporting limit to ensure that all data was reported in a standardised format (Table 6, Table 7), (EFSA, 2016).

This approach was also adopted for the UK samples. Therefore, in some cases, the true calculated reporting limits were lower than those reported for EFSA samples. For a small number of the additional UK samples low levels of TAs were measured at a concentration below the assigned reporting limit. These results are presented in parenthesis in the tables and have been reported for information only. For some matrices, for example, some single grain flours, and the black and green tea samples, it was necessary to increase the reporting limit as there was a significant background that reduced the signal to noise ratio, or the recovery was low. These are also highlighted in the results tables (Table 23 to Table 27).

12. Results and Discussion

12.1. Method Validation Results – Cereals

Initial validation was completed and the data from this validation highlighted several problems, including problems with chromatography for some analytes, interferences in the chromatograms for some analytes, with poor peak shapes and variable LOQs for some matrices, buckwheat being the one of worst. It was concluded that using matrix matched calibration standards did not improve the analysis, unless the matrix was exactly the same as sample under test.

Changes were made to the initial protocol to add a methanol wash during SPE, increase the ammonia content of the SPE elution solvent and to modify the chromatography conditions to use ammonium carbonate in the mobile phase. It was found that the pH is critical to the chromatographic performance for these analytes and best performance was obtained when the mobile phase was maintained at pH 10 or higher.

The validation was repeated and full results are given in the Validation Report attached as Annex 3.

Recovery was determined from spiking samples before extraction and clean-up. This however also has the drawback of including any matrix effects (suppression or enhancement) and therefore the recovery value reported is not a ‘true’ recovery. The actual recovery of the procedure can be measured by comparison of the Matrix Matched Recovery samples (MMRS) samples, which were spiked after extraction and clean-up, with samples spiked before extraction and clean-up. The MMRS samples were spiked at a level of 10 µg/kg. The summary of these results is given in Table 6 as well as the standardised LOD and LOQ values used to report data to EFSA.

Table 6. Summary of Fera average recovery and RSDs and standardised LODs and LOQs of individual TAs at 10 µg/kg in single flours and cereal-based products (breakfast cereals, bread, biscuits and buckwheat flour) during in-house validation

Compound name	FERA (n = 4)		FERA	
	Recovery (%)	RSD recovery (%)	LOD (µg/kg)	LOQ (µg/kg)
Low molecular weight TAs				
6-Hydroxytropinone	86	7	0.5	2.5
Nortropinone	82	16	0.5	5.0
Pseudotropine	87	4	0.5	5.0
Scopine	87	3	0.5	2.5
Scopoline	80	8	0.5	5.0
Tropine	88	5	0.5	2.5
Tropinone	59	28	0.5	2.5
Convolvulaceae-type TAs				
Convolamine	114	20	0.1	0.5
Convolidine	88	15	0.25	1.0
Convolvine	133	21	0.25	1.0
Fillalbine	89	12	0.1	0.5
Datura-type TAs				
O-Acetylscopolamine	16[(b)]	39[(b)]	0.1	0.5
Anisodamine	94	10	0.1	0.5
Anisodine	100	12	0.1	0.5
Apoatropine	99	22	0.1	0.5
Aposcopolamine	118	15	0.1	0.5
Atropine[(a)]	95	16	0.05	0.5
Homatropine	99	12	0.1	0.5
2α-Hydroxymethyl atropine	93	5	0.1	0.5
Littorine	126	25	0.1	0.5
Noratropine	93	17	0.1	0.5
Norscopolamine	102	19	0.1	0.5
Phenylacetoxytropane	91	28	0.1	0.5
Scopolamine [(a)]	105	9	0.05	0.5

Overall the method performance was satisfactory for atropine and scopolamine, as the internal standard used for these compounds corrects for matrix effects. In all cases the performance criteria for recovery and repeatability from Regulation (EC) No 401/2006 were met except for muesli and bread where the average recovery for bread matrix spiked at 1 µg/kg scopolamine which was outside the 60 to 120% recommended range. In all cases the MMRS recovery was acceptable.

The apparent recovery values observed for the other analytes were much improved compared to the initial validation analyses, however there were instances of low recovery. However when the MMRS recovery values were considered, these values for the most part, were within the acceptable range, although tropinone and acetylscopolamine recovery was below 50% for several products. Buckwheat flour was a problematic matrix with very low apparent recovery for many analytes, although true recovery from MMRS spikes was acceptable.

In all cases the repeatability of the method was very good with a maximum RSD (within batch variability) of 13% observed, even at the low spiking level of 1 µg/kg. This is well within the performance criteria from Regulation (EC) No 401/2006. The validation of all four partner laboratories is presented in the EFSA report and there is excellent agreement between the laboratories.

12.2. Method Validation Results - Teas

The final SOP (Annex 2) also includes the modified procedure for teas. The modification was to load half the volume of sample extract onto the SPE cartridge. The concentration of the required calibration standard solutions was therefore also different. The validation plan used was as described in section 11.3. A copy of the Fera validation report for teas is given as Annex 4.

Although less sample extract was cleaned-up before analysis the tea samples contained numerous interferences in the chromatograms, particularly for the lower m/z tropanes. In some cases (tropine, scopoline and convolvine) these interferences, or a high background, affected the analyte peak such that it was necessary to quantify using a different transition to that specified in the SOP. In cases where interferences could not be avoided and affect quantification of the spikes, the spike concentrations have been corrected by subtraction of the calculated concentration in the associated blank. In the case of nortropinone, an interference made it impossible to quantify the spikes at 1 µg/kg.

A summary of the recovery data obtained for the 10 µg/kg spikes as well as the standardised LODs and LOQs for Fera are given in Table 7. Validation data for all partners is given in the EFSA report, the data produced by all laboratories was very consistent.

Table 7. Average recovery and RSD of individual TAs at 10 µg/kg in dry (herbal) teas (matrices: green tea, peppermint tea, mixed herbal tea) obtained during in-house validation. LOD and LOQs calculated for standardised reporting to EFSA.

Compound name	FERA[(a)] Recovery (%)	LOD (µg/kg)	LOQ (µg/kg)
Low molecular weight TAs			
6-Hydroxytropinone	46	1.0	5.0
Nortropinone	36	2.5	5.0
Pseudotropine	80	2.5	5.0
Scopine	74	1.0	5.0
Scopoline	75	1.0	5.0
Tropine	82	2.5	5.0
Tropinone	38	1.0	5.0
Convolvulaceae-type TAs			
Convolamine	32	0.5	1.0
Convolidine	58	0.5	1.0
Convolvine	40	0.5	1.0
Fillalbine	48	0.5	1.0
Datura-type TAs			
O-Acetylscopolamine	20	0.25	0.5
Anisodamine	83	0.25	0.5
Anisodine	84	0.25	0.5
Apoatropine	38	0.25	0.5
Aposcopolamine	58	0.25	0.5
Atropine[(c)]	89	0.1	0.5
Homatropine	69	0.25	0.5
2α-Hydroxymethyl atropine	76	0.25	0.5
Littorine	67	0.25	0.5
Noratropine	75	0.25	0.5
Norscopolamine	88	0.25	0.5
Phenylacetoxytropane	53	0.25	0.5
Scopolamine[(c)]	89	0.1	0.5

(a): Single-day validation using green tea.

QC samples were also run with each batch of tea samples. These QC consisted of blank green tea, a sample of this green tea spiked at 10 µg/kg at Fera, a sample spiked at 10 µg/kg at RIKILT and a blank extract spiked at a level equivalent to 10 µg/kg after extraction and clean-up (i.e. MMRS). In addition, an MMRS was also prepared for each of the eight different types of tea which were sampled. These were used to calculate the actual recovery by comparison with the same sample spiked before extraction and clean-up. This actual recovery takes into account the whole analytical procedure and accounts for any matrix suppression or enhancement effects that occur in the mass spectrometer. Therefore it is a real indication of the performance of the method. Lemon balm gave the lowest recoveries of all the products tested in the validation with recoveries in the range 23 - 74%. Most alkaloids gave acceptable recovery performance, however the low molecular weight tropane alkaloid compounds tended to have lower recovery for all sample types. Repeatability was excellent for all analytes at all levels in all matrices tested with RSD values ranging from <1% to 19% for 6 replicate analyses at any individual level, even where recovery values were low.

The large variation in "recovery" values for the same analyte in different types of tea highlights that it would not be possible to accurately quantify residues in a particular tea using matrix extracted standards in a different type of tea. For this reason, survey samples were analysed using solvent calibration standards, with each sample analysed alongside an overspike to allow individual recovery values to be calculated.

12.3. Method validation Results - Herbal tea infusions

Validation of the procedure for the preparation of herbal tea infusions and analysis of the infusions was only conducted at RIKILT who carried out all analyses of tea infusions. The validation focussed on the extraction of the TAs from the tea infusion. However, the efficiency of extraction of TAs from the solid tea during tea preparation was also taken into account. Low LODs and LOQs could be achieved for many of the *Convolvulaceae*-type and *Datura*-type TAs. For the low MW TAs the LODs and LOQs were higher, but this was in accordance with the results obtained for the dry teas. Recoveries from tea infusion varied between 70 and 100% with acceptable repeatability for most TAs, except for a few low MW TAs. The efficiency of extraction of TAs from dry tea during tea preparation was between 60 and 85% for most TAs. Exceptions were the *Convolvulaceae*-type TAs which were extracted with lower (50-60%) efficiency and apoatropine that had an extraction efficiency of 43%. This compound is relatively lipophilic that may not be extracted well with (hot) water. The low MW TAs were extracted efficiently, indicating that evaporation from the hot infusion is likely to be negligible.

12.4. Method performance

The method was less robust for some of the low molecular weight tropane alkaloids that elute very early in the chromatography run. Due to the early elution time these compounds are more prone to poor peak shape and subject to interferences which makes quantification difficult in some cases. If in the future it is decided these compounds are significant or need to be controlled then it may be appropriate to consider developing a separate targeted method for them, perhaps using Hydrophobic Interaction Liquid Chromatography (HILIC). The current method, as it is a multi-toxin method that covers a broad range of molecular weights and polarity and pKa values, is by necessity a compromise as it cannot be optimised for all analytes as they are chemically different. It was suitable for this large survey to give an insight into the levels of tropane alkaloids and the high level of QC analyses included with the survey gives a high degree of confidence for the results reported.

12.5. Method validation results – calystegines

The method validation for calystegines was carried out at RIKILT and the two other partner laboratories. RIKILT validation results for the potato and aubergine method validation are are shown in Table 8, as all UK samples for calystegines were analysed by RIKILT. Good recoveries (between 83 and 98%) were obtained for the calystegines. On the basis of these results it was decided to report the results without correction for the recovery.

Table 8. Method validation results (recovery as extraction efficiency[(a)]) and LOD and LOQ for calysegines in potato and aubergine

Compound name	LOD	LOQ	Recovery potato	RSD potato	Recovery aubergine	RSD aubergine
	(mg/kg)	(mg/kg)	(%)	(%)	(%)	(%)
Calystegine A_3	0.5	1.0	93.8	3.9	98.0[(b)]	-
Calystegine A_5	1.0	2.5	100[(c)]	-	-	-
Calystegine B_1	0.25	1.0	89.0[(a)]	-	89.8	0.8
Calystegine B_2	0.25	1.0	91.7	3.3	90.4	0.7
Calystegine B_3	0.25	1.0	-	-	-	-
Calystegine B_4	0.25	1.0	92.7	4.2	-	-

- No data
(a) based on incurred materials
(b) n = 1
(c) n = 3

13. Sample results – EFSA samples

The full results of the EFSA samples were submitted to EFSA and FSA in the EFSA SSD spreadsheet format. The results were reported in a standardised format for LOD and LOQ to allow consistency within the overall data set. Overall in the EFSA survey 1305 samples were analysed for the 24 TAs, and a further 404 samples were analysed for calystegines. In total 227 UK samples (197 cereal products, 10 green beans and vegetables and 20 teas) were analysed for TAs for the EFSA survey. Sixty-nine UK samples (48 cereal products, 12 oilseeds and 9 teas) were analysed as additional UK samples. A summary of the TA results for the UK samples analysed at Fera for the EFSA survey is given in Table 9. The full results of all UK EFSA samples are given in Table 12 to 27. In addition 44 samples of UK retail potatoes and 15 samples of UK retail aubergines were analysed for calystegines by RIKILT for the EFSA survey, so 286 UK samples were analysed in total.

13.1. Cereal products results

At least one sample of each of the cereal food categories was found to contain tropane alkaloids, although in many cases the levels measured were extremely low. Buckwheat contained the fewest residues with only 1 sample out of 18 found to contain very low levels of atropine and scopolamine (<1 µg/kg total) (individual results in Table 12).

13.1.1. Breakfast cereal results

Breakfast cereals were also found to have infrequent low levels of tropane alkaloids, with only 2 out of 30 samples containing alkaloids (Table 13). Atropine and scopolamine were the only alkaloids found. The highest levels found were 0.67 µg/kg atropine and 0.38 µg/kg scopolamine in sample S15-100597, a high bran cereal.

13.1.2. Maize product results

Maize products showed a similar contamination pattern, with 2 out of 13 products found to contain alkaloids, but only atropine and scopolamine were detected (Table 14). The sample that contained the highest level of 0.63 µg/kg atropine and 0.16 µg/kg scopolamine was a corn meal product from an ethnic supermarket (S16-003305).

13.1.3. Bread and pasta results

Only 5 out of 33 bread and pasta samples contained tropane alkaloids, although in addition to atropine and scopolamine, one other alkaloid (anisodamine) was also detected. The highest level of any alkaloid found in these products was 0.15 µg/kg, with no sample above 1 µg/kg total alkaloids (Table 15).

13.1.4. Biscuits and baked goods results

Biscuits and baked goods were also found to contain alkaloids, although again at very low levels (Table 16). In total 5 out of 32 samples contained detectable alkaloids, atropine was found in 5 samples and scopolamine was also detected in two of those samples. The highest level found was 1.23 µg/kg atropine and 0.65µg/kg scopolamine in sample S15-100636, a sample of buckwheat galettes (pancakes).

13.1.5. Millet, sorghum and other flours results

Millet, sorghum and other flours contained the broadest range of tropane alkaloids, with one sample containing up to 11 different alkaloids. This was sample S16-

003153, a sample of yellow millet supplied by an on-line retailer. This sample contained 9.8 µg/kg atropine and 12.9 µg/kg scopolamine in addition to 8 other alkaloids. The total alkaloid content was 33.6 µg/kg. Overall 3 out of 25 samples of millet and other cereals contained tropane alkaloids (Table 17).

Table 9. Summary of alkaloids found and highest concentration levels measured in EFSA survey samples

Product type	Total samples	No samples positive	Conc. Range (µg/kg)	Max. No alkaloids per sample	Alkaloids found and highest levels (µg/kg)
Biscuits & Baked goods	32	5	0.05 to 1.23	2	Atropine (1.23) Scopolamine (0.65)
Buckwheat	18	1	0.09 to 0.56	2	Atropine (0.56) Scopolamine (0.09)
Millet & sorghum	25	3	0.12 to 12.9	11	Anisodamine (0.74) Apoatropine (0.49) Aposcopolamine (0.25) Atropine (9.8) Noratropine (0.12) Norscopolamine (0.73) Phenylacetoxytropane (0.01) Pseudotropine (2.17) Scopoline (4.72) Scopolamine (12.9) Tropine (2.26) Tropinone (0.78)
Infant foods	46	14	0.10 to 773	8	Atropine (3.73) Convolamine (1.30) Convolidine (13.4) Fillalbine (28.3) 6-Hydroxytropine (1.49) Pseudotropine (44.1) Scopolamine (0.51) Tropine (773) Tropinone (2.43)
Bread & pasta	33	5	0.05 to 0.15	2	Anisodamine (0.13) Atropine (0.13) Scopolamine (0.15)
Maize products	13	2	0.07 to 0.63	2	Atropine (0.63) Scopolamine (0.16)
Breakfast cereals	30	2	0.32 to 0.67	2	Atropine (0.67) Scopolamine (0.38)
Herbal teas	20	16	0.28 to 129	8	Anisodamine (3.33) Apoatropine (0.32) Aposcopolamine (0.28) Atropine (129) Convolidine (0.78) Convolvine (19.1) Noratropine (0.43) Norscopolamine (0.98) Pseudotropine (9.26) Scopolamine (34.1) Tropine (14.0) Tropinone (2.96)
Beans & veg	10	3	0.64 to 571	6	Convolamine (0.70) Convolidine (6.41) Fillalbine (11.6) Pseudotropine (43.6) Tropine (571) Tropinone (1.05)

13.1.6. Infant foods results

Of the 46 infant foods analysed for this part of the survey 14 were found to contain tropane alkaloids, with some samples containing as many as 8 different alkaloids (Table 18). Seven samples were found to contain atropine above the reporting limit of 0.05 µg/kg, with three of these above the regulatory limit of 1 µg/kg laid down in Regulation (EU) No 339/2016 (3). Five samples contained detectable residues at very low levels below the reporting limit, but these have not been reported as positive findings due to the higher uncertainty associated with these results. The highest level of atropine found was 3.73 µg/kg in a sample of fruit crunch cereal (S15-100580). This sample also contained 0.51 µg/kg scopolamine, which was the highest level of scopolamine found in these products.

Four samples contained tropine at concentrations ranging from 109 to 773 µg/kg. These samples also contained several other alkaloids, mainly the convolamine type indicating the source was *Convolvulaceae*-type plants. Sample S16-003181 contained the highest level of tropine (773 µg/kg), it also contained 1.25 µg/kg atropine as well as convolamine, convolidine, fillalbine, pseudotropine, scopolamine and tropinone. A similar pattern was also seen in sample S15-100583. These products both contained millet as an ingredient, but also contained vegetables including red pepper, which was a food type identified in the literature search as a possible source of alkaloids.

It should be noted that all baby food samples were analysed ‘as received’. In some cases the products would be reconstituted with water or milk before consumption. The samples that contained the highest levels of alkaloids, including those that exceeded the regulatory limit for atropine, were all dried products that would be reconstituted before consumption.

13.2. Vegetable results

Only 10 samples of green beans and stir fry mixes were analysed in the survey as these were considered to be a low risk source of alkaloids in the diet. Three of the vegetable samples were found to contain alkaloids although none contained atropine or scopolamine (Table 19). The highest level found was 571 µg/kg tropine in SS16-003258 a sample of frozen stir fry vegetables. The sample also contained convolamine, convolidine, fillabine, pseudotropine and tropinone although at much lower levels than tropine. Two other samples showed similar patterns of alkaloid occurrence, but the tropine levels were much lower at 24.1µg/kg (S16-003262) and 27.9 µg/kg (S16-003264), both were stir fry mixes. None of the green beans contained tropane alkaloids.

13.3. Herbal tea results

13.3.1. Dry herbal tea results

The tea samples were the products that were most frequently found to contain tropane alkaloids (Table 20). Twenty herbal tea samples were analysed, 16 were found to contain some tropane alkaloids. Of the 16 samples containing TAs, 8 contained scopolamine and 10 contained atropine above the reporting limit of 0.1 µg/kg each. Several other alkaloids were also found in the samples. One sample of peppermint tea contained eight different alkaloids. This sample (S16-003243) contained 50.2 µg/kg atropine and 34.1 µg/kg scopolamine. The highest level of atropine measured was 129 µg/kg in sample S16-003246, a peppermint tea. This sample also contained 33.7 µg/kg scopolamine and five other alkaloids (anisodamine, apoatrpoine, noratropine, norscopolamine and tropine) at low concentrations. These concentrations were measured in the dry tea from the tea bags.

It must be noted that the analysis of the teas was quite difficult particularly for tropine and tropinone. There were interferences in the chromatograms and for some analytes the third choice of transition had to be used to quantify the analyte as the other transitions contained interferences. This resulted in higher reporting limits (LOD and LOQ) for these products than some of the other samples tested in the survey (Table 7). Some of the tropine results were not confirmed as either it was not possible to determine two transitions to calculate an ion ratio to confirm the results or because the ion ratio of two transitions failed as one contained an interference. The levels reported for tropine ranged from 1 to 14 µg/kg, but only one result was confirmed. Results for atropine and scopolamine were not affected as internal standards were used.

As part of the EFSA study, Fera was asked to carry out some confirmation analyses for some samples of tea initially analysed by VSCHT, Czech Republic. They had found high levels of alkaloids, particularly convolvine, at concentrations up to 8 mg/kg. RIKILT had not been able to confirm these results and Fera was asked to carry out third party analysis to confirm the results. The results Fera found were more aligned to the VSCHT results, although the highest level found was 4074 µg/kg convolvine in the sample that VSCHT had reported >8000 µg/kg. This sample also contained several other alkaloids including tropine, atropine and scopolamine. Most alkaloid levels were in good agreement between the three labs, it was only the convolvine concentration that varied. The reason for the discrepancy between results is still not clear but could be due to inefficient extraction or saturation / overloading of the clean-up columns. Fera’s results were included in the SSD spreadsheet submitted to EFSA, and are reported in the EFSA survey report (Mulder et al, 2016).

13.3.2. Results for herbal tea infusions

RIKILT studied the efficiency of the transfer of TAs from herbal tea to the tea infusion by using a selection of contaminated dry herbal teas. Twenty herbal teas from all partners were selected that contained the highest TA content and in which at least one TA was present at 10 µg/kg. Detailed results of the infusions from UK samples were not reported separately to Fera. The results of the infusions are reported as collated data in the EFSA report as a percentage of the concentration reported for the dry tea (page 56, Figure 12, Mulder et al, 2016). Tea infusions were prepared using a standardised protocol (ISO 3103, ISO 1980) (Section 11.5). The results obtained for the tea infusions were compared with those obtained for the corresponding dry teas, to calculate the extraction efficiency of the boiling water. A relatively wide variation was observed, however it was concluded that in general the extraction from the dry tea by infusion was less than 100% (or more precisely: less than 100% compared to the solvent extraction procedure used for dry tea). Transfer efficiency was highly variable, but on average a transfer of 47% was obtained for the 20 teas included in the study across all TAs measured. Atropine and scopolamine were both present in 19/20 of these samples and for these two compounds the average transfer efficiency was 54% and 42% respectively. Higher average (70%) extraction efficiency for infusion preparation had been derived for the TAs, including atropine (71%) and scopolamine (84%) during the initial method validation. However this was performed with dry tea samples that were spiked with TA standards before infusion preparation. The transfer from the incurred survey samples was lower as the TAs are bound within the tea matrix, whereas for the spiked samples the TAs are on the surface of the tea leaves.

The highest level of atropine found in a UK tea sample was 129 µg/kg. If it is presumed a tea bag is 2g, and that approximately 50% of the atropine is extracted during infusion preparation then a drink prepared from this material would contain 129 ng of atropine. Due to the heterogenous nature of the contamination this will vary but it gives an indication of likely intake from this product.

13.4. Calystegine results – Aubergines and potatoes

A summary of calystegine results is given in Table 10. All samples contained some detectable calystegines. Calystegine A3, calystegine B2 and calystegine B4 were the calystegines predominantly detected in potatoes. Calystegine A3 was detected at the highest concentration (max 309.9 mg/kg), followed by calystegine B2 and calystegine B4. Calystegine A5 was present in a small number of samples (6 samples) at low levels. Calystegine B3 was not detected in any of the UK potato samples. The full results for potatoes are given in Table 21.

Calystegine A3, calystegine B1 and calystegine B2 were detected in aubergines, while calystegine A3, calystegine A5 and calystegine B4 were not detected in UK bought samples. Calystegine B2 was detected at the highest concentration in aubergine, the highest level found was 124.8 mg/kg (Table 22).

Table 10. Summary of calystegine results found for EFSA potato and aubergine samples

Product type	**Total samples**	**No samples positive**	**Conc. Range (mg/kg)**	**Max. No alkaloids per sample**	**Alkaloids found and highest level (mg/kg)**
Aubergines	15	15	0.5 – 124.8	3	Calystegine A3 (28.1) Calystegine B1 (28.6) Calystegine B2 (124.8)
Potatoes	44	44	0.5 – 309.9	4	Calystegine A3 (309.9) Calystegine A5 (10.6) Calystegine B2 (127.1) Calystegine B4 (16.7)

13.5. Cereal products Results – UK samples

A summary of the FSA survey sample results is given in Table 11.

13.5.1. Single grain flour results

Five single grain flours purchased in retail stores did not contain detectable tropane alkaloids. The samples were wheat flour, rye and spelt (Table 23).

Seventeen samples were collected and supplied by nabim (National Association of British and Irish Millers). These were mainly wholemeal wheat flour, but also included one buckwheat and one millet flour. Samples for use in ethnic foods were requested and two samples were specifically labelled as chapatti flour. Most of the samples were of UK origin but the buckwheat and millet were from China, and two wheat flour samples were labelled as produced from grain from Kazhakstan & Russia. No alkaloids were detected in any of the samples above the reporting limits (Table 24).

13.5.2. Maize products results

Six maize products were analysed, of these 2 contained low levels of tropane alkaloids. One sample of polenta (S15-100709) contained 0.22 µg/kg atropine and 0.06 µg/kg scopolamine, and a sample of gluten free pasta (S15-100708) contained a very low level of atropine (0.05 µg/kg) (Table 23).

Table 11. Summary of alkaloids found and highest concentration levels measured in FSA survey samples

Product type	Total samples	No samples positive	Conc. Range (µg/kg)	Max. No alkaloids per sample	Alkaloids found and highest levels (µg/kg)
Nabim flours	17	0	-	0	-
FSA Infant foods	20	9	0.05 to 492	8	Aposcopolamine (0.08) Atropine (1.14) Convolamine (1.03) Convolidine (8.84) Fillalbine (38.6) Nortropinone (4.53) Pseudotropine (29.6) Scopolamine (0.88) Tropine (492) Tropinone (1.66)
FSA flours and maize products	11	2	0.05 to 0.22	2	Atropine (0.22) Scopolamine (0.06)
Oilseeds	12	1	0.09 to 0.11	2	Atropine (0.11) Scopolamine (0.09)
Black & green teas	9	2	0.05 to 0.15	1	Scopolamine (0.15)

13.5.3. Infant foods results

Twenty samples of infant foods were analysed, of these 9 samples were found to contain tropane alkaloids with several samples containing multiple alkaloids (Table 25). The sample that contained the highest level of tropane alkaloids was S15-100702, a cereal based savoury meal that contained millet and vegetables including red pepper. In fact, it was the same product that was found to contain the highest levels for the EFSA samples. It contained 1.14 µg/kg of atropine, 0.26 µg/kg scopolamine as well as 492 µg/kg tropine and several other alkaloids (8 in total). As noted above all samples were analysed 'as received', the samples with the highest levels of alkaloids would be reconstituted before consumption.

A sample of ready to eat Thai curry (S16-003292) contained 7 alkaloids, including 97.8 µg/kg tropine, as well as tropinone, pseudotropine, fillalbine, convolamine, convolidine and a trace amount of atropine. Three other ethnic baby foods also contained low levels of varying amounts of convolidine, nortropinone, pseudotropine, tropine and tropinone. The highest level of scopolamine (0.88µg/kg) was found in a sample of a savoury cereal based product that also contained vegetables and millet (S16-003294). The cereal based products were sampled to contain millet or sorghum as an ingredient, however there appeared to be a consistent pattern that the breakfast cereal or products containing fruit did not contain tropane alkaloids, whereas the savoury or main meal products that contained vegetables did contain tropane alkaloids. Also, all four samples of ready to eat ethnic meals contained detectable alkaloids, these were vegetable based, although some did contain meat, but had little if any cereal ingredients. This could suggest that the contribution to the alkaloid content comes from the vegetable ingredients and not the cereal component. Only one sample exceeded the EU maximum level for atropine, this was sample S15-100702, which was just above the 1 µg/kg limit.

13.6. Oilseeds results

Twelve samples of oilseeds or oilseed containing products were analysed, and only one was found to contain very low levels of atropine (0.11 µg/kg) and scopolamine (0.09 µg/kg) (Table 26). This was sample S16-001242 which was a sample of organic flaxseed (linseed) from Canada.

13.7. Black and green tea results

Nine samples of black (5 samples) and green (4 samples) of tea were analysed (Table 27). Two samples contained low levels of scopolamine, one at 0.15 µg/kg (black tea S16-003249) and one at 0.05 µg/kg (black tea, S16-003241) although this level was below the reporting limit of 0.1 µg/kg and could not be confirmed by ion ratio.

14. Conclusions

A comprehensive literature review was completed. This highlighted possible sources of tropane alkaloids in foods, and foods likely to be contaminated with TAs. This information was used to develop the sampling plans used for the EFSA and FSA surveys. Sources of contamination were identified as *Datura* type seeds in some products and the increase of *Convolvulus* type weeds in fields of food crops. Calystegines were also included in the review. These are inherent toxins found in mainly *Solanaceae* plants. Potatoes and aubergines were included in the sampling plan as the major source of these compounds in the diet

Food types identified as being the most important to sample were cereal based foods including those that contain millet, sorghum and poppy seed; infant foods, (containing millet, sorghum); maize products, single grain flours, especially buckwheat; canned or frozen green beans and vegetable mixes and herbal teas. Oilseeds, black & green tea, some cereal products and infant food, in particular ethnic infant food or infant food containing millet were sampled for the UK only survey. Potatoes and aubergines were included for calystegine analysis.

The review concluded very little is known about the toxicity of TAs other than atropine and scopolamine. Therefore as many TAs as possible should be included in the analytical method. TAs for which reliable analytical standards are available were included to increase the knowledge base about the occurrence of these compounds in foods.

Method validation was carried out for cereals and teas. Overall the method is satisfactory for most analytes, the validation showed recovery met the required criteria, and the method had good repeatability. The method has been demonstrated to meet the required performance criteria for atropine and scopolamine by the results of the validation exercise and QC data generated during the survey and participation using this method in a PT run by the EURL for atropine and scopolamine gave 100% satisfactory results.

A total 227 UK samples (197 cereal products, 10 green beans and stir fry vegetables and 20 teas) were analysed for TAs for the EFSA survey. Green beans and vegetable stir fry mixes contained TAs at levels from <1µg/kg up to 571 µg/kg tropine, up to 6 TAs were found in individual samples.

An additional 52 UK retail samples (20 cereal based infant foods, 11 single grain flours and maize products, 12 oilseeds and 9 teas), and 17 single grain flours sampled directly at mills were analysed for TAs on behalf of the FSA. Overall the TA levels were low in the additional UK samples. Of 11 cereal based foods only 2 contained very low levels of TAs. Oilseeds and green & black tea showed similar results, only 1 out of 12 and 2 out of 9 samples respectively contained TAs at trace levels. UK milled flours (17 samples) did not contain any TAs. Infant foods were the category where most TAs were detected, 9 out of 20 samples contained TAs, with up to 8 TAs measured in individual samples. One sample contained atropine at 1.14 µg/kg.

For the breakfast cereals 2 out of 30 samples contained alkaloids, only atropine and scopolamine were detected. The highest levels found were 0.67 µg/kg atropine and 0.38 µg/kg scopolamine in a high bran cereal. Only 2 out of 13 maize products contained TAs, again only atropine and scopolamine were detected. For bread and pasta only 5 out of 33 samples contained TAs, as well as atropine and scopolamine one other alkaloid (anisodamine) was also detected. A small number of biscuits and baked goods contained alkaloids at very low levels. In total 5 out of 32 samples contained atropine and scopolamine was also detected in 2 of those samples. The highest level found was 1.23 µg/kg atropine and 0.65µg/kg scopolamine in buckwheat galettes (pancakes). Millet, sorghum and other flours contained the broadest range of tropane alkaloids, with one sample containing up to 10 different alkaloids. This was a sample of yellow millet that contained 9.8 µg/kg atropine and 12.9 µg/kg scopolamine as well as 8 other alkaloids. The total TA content was 33.6 µg/kg. Overall 4 out of 25 samples of millet and other cereals group contained tropane alkaloids.

Cereal based infant foods are the only products included in this survey where maximum levels of 1 µg/kg each for atropine and scopolamine are in force (3). Four out of 66 UK samples were found to exceed these levels in this survey, (3/46 for the EFSA survey and 1/20 for the FSA survey). The highest level found was 3.73 µg/kg atropine.

All the samples that exceeded the limit were dry products, they were analysed as received but would be reconstituted before consumption. These products contained vegetables, including pepper, as well as cereal and it is likely these were the source of the TAs. Vegetables from the *Solanaceae* family were identified in the literature review as a possible source of tropane alkaloids. Several infant foods contained high levels of tropine (up to 773 µg/kg), and up to 8 different alkaloids were also found in some samples. The results from some of the vegetable samples analysed (high tropine levels) would support this. The relevance of the other TAs measured is hard to evaluate due to the lack of toxicity data about these compounds.

Herbal teas were found to be commonly contaminated with TAs. Sixteen out of 20 teas from the UK contained detectable TAs. The highest level found was 129 µg/kg atropine in a peppermint tea sample. Tea infusions were prepared from a selection of teas and analysed for TAs at RIKILT. On average it was found that approximately 47% of the TAs present in naturally incurred dry tea transferred to the infusion.

UK purchased samples of potatoes and aubergines were analysed for calystegines at RIKILT. Forty four samples of potatoes and 15 samples of aubergines were analysed. The two vegetables showed different contamination patterns. Levels in potatoes were on the whole higher than aubergines. Calystegine A3, calystegine B2 and calystegine B4 were predominantly detected in potatoes. The highest level found was 310 mg/kg calystegine A3. Calystegine A3, calystegine B1 and calystegine B2 were detected more frequently in aubergines. Calystegine B2 was detected at the highest concentration in aubergine, the highest concentration measured was 124.8 mg/kg.

15. References

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16. Acknowledgements

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Table 12. Tropane alkaloid results for EFSA buckwheat samples, µg/kg.

		Acetyl scopolamine	Anisodamine	Anisodine	Apo tropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.1	0.1	0.1	0.1	0.1	0.05	0.1	0.25
Sample code	Product description	Conc (µg/kg)							
S15-100555	Wholemeal Buckwheat Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100556	Gluten Free Buckwheat Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100557	Buckwheat Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100558	Buckwheat	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100559	Organic Buckwheat Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100560	Buckwheat Flour organic	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100561	Buckwheat Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100563	Organic Gluten Free Sprouted Whole Buckwheat Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100564	Buckwheat Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100565	Organic Gluten Free Sprouted Whole Buckwheat Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003147	Buckwheat Flour organic	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003148	Buckwheat Flour	<0.1	<0.1	<0.1	<0.1	<0.1	0.56	<0.1	<0.25
S16-003152	Buckwheat Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003158	Buckwheat Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003160	Roasted Buckwheat	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003234	Buckwheat Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-015536	Organic Buckwheat	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-015548	Buckwheat Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25

Table 12. Tropane alkaloid results for EFSA buckwheat samples, µg/kg (contd.)

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.25	0.1	0.1	0.1	0.5	0.1	0.1	0.1
Sample code	Product description	Conc (µg/kg)							
S15-100555	Wholemeal Buckwheat Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100556	Gluten Free Buckwheat Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100557	Buckwheat Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100558	Buckwheat	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100559	Organic Buckwheat Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100560	Buckwheat Flour organic	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100561	Buckwheat Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100563	Organic Gluten Free Sprouted Whole Buckwheat Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100564	Buckwheat Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100565	Organic Gluten Free Sprouted Whole Buckwheat Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003147	Buckwheat Flour organic	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003148	Buckwheat Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003152	Buckwheat Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003158	Buckwheat Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003160	Roasted Buckwheat	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003234	Buckwheat Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-015536	Organic Buckwheat	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-015548	Buckwheat Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1

Table 12. Tropane alkaloid results for EFSA buckwheat samples, µg/kg (contd.)

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	0.5	0.25	0.5	0.5	0.5	0.05	0.5	0.5
Sample code	Product description	Conc (µg/kg)							
S15-100555	Wholemeal Buckwheat Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100556	Gluten Free Buckwheat Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100557	Buckwheat Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100558	Buckwheat	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100559	Organic Buckwheat Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100560	Buckwheat Flour organic	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100561	Buckwheat Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100563	Organic Gluten Free Sprouted Whole Buckwheat Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100564	Buckwheat Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100565	Organic Gluten Free Sprouted Whole Buckwheat Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003147	Buckwheat Flour organic	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003148	Buckwheat Flour	<0.5	<0.25	<0.5	<0.5	<0.5	0.09	<0.5	<0.5
S16-003152	Buckwheat Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003158	Buckwheat Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003160	Roasted Buckwheat	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003234	Buckwheat Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-015536	Organic Buckwheat	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-015548	Buckwheat Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5

Table 13. Tropane alkaloid results for EFSA breakfast cereal µg/kg

		Acetyl scopolamine	Anisodamine	Anisodine	Apo atropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.1	0.1	0.1	0.1	0.1	0.05	0.1	0.25
Sample code	Product description	Conc (µg/kg)							
S15-100597	High Bran cereal	<0.1	<0.1	<0.1	<0.1	<0.1	0.67	<0.1	<0.25
S15-100599	Wheat flake biscuits	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100600	Four grain Porridge	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100601	Toasted Muesli with Buckwheat	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100602	Wheat breakfast cereal	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100603	Oatbran flakes Very Berry	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100604	Pumpkin & mango granola	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100605	Wholegrain Wheat bitesize	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100606	Bircher Muesli base	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100607	Maple Sunrise	<0.1	<0.1	<0.1	<0.1	<0.1	0.32	<0.1	<0.25
S15-100608	Scottish Porridge	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100609	Organic Muesli	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100610	Crispy G/F Buckwheat Flakes Organic	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100611	Buckwheat Puffs G/F Organic	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100613	Wheatflake biscuits Organic	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100614	Sorghum gluten free cereal	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003190	Wheat Bisks	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003191	Original rice and wheat flakes	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003193	Crunchy Spelt Bran Flakes	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003195	Wholegrain Wheat breakfast cereal	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003196	Organic Millet Puffs wholegrain	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25

Table 13. Tropane alkaloid results for EFSA breakfast cereal µg/kg (contd.)

		Acetyl scopolamine	Anisodamine	Anisodine	Apo atropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.1	0.1	0.1	0.1	0.1	0.05	0.1	0.25
Sample code	Product description	Conc (µg/kg)							
S16-003197	Organic Millet Flakes wholegrain	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003198	Organic Oats	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003199	Organic Express Porridge sachets	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003200	Organic Porridge Chunky Traditional	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003201	Buckwheat toasted muesli	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003202	Organic Granola	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003203	Muesli, puffed spelt, pumpkin, sunflower, flax seeds & sultanas	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003204	Muesli	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003205	Muesli, Quinoa, apple, cranberry & chia seeds	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25

Table 13. Tropane alkaloid results for EFSA breakfast cereal µg/kg (contd.)

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.25	0.1	0.1	0.1	0.5	0.1	0.1	0.1
Sample code	Product description	Conc (µg/kg)							
S15-100597	High Bran cereal	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100599	Wheat flake biscuits	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100600	Four grain Porridge	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100601	Toasted Muesli with Buckwheat	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100602	Wheat breakfast cereal	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100603	Oatbran flakes Very Berry	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100604	Pumpkin & mango granola	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100605	Wholegrain Wheat bitesize	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100606	Bircher Muesli base	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100607	Maple Sunrise	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100608	Scottish Porridge	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100609	Organic Muesli	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100610	Crispy G/F Buckwheat Flakes Organic	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100611	Buckwheat Puffs G/F Organic	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100613	Wheatflake biscuits Organic	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100614	Sorghum gluten free cereal	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003190	Wheat Bisks	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003191	Original rice and wheat flakes	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003193	Crunchy Spelt Bran Flakes	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003195	Wholegrain Wheat breakfast cereal	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003196	Organic Millet Puffs wholegrain	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1

Table 13. Tropane alkaloid results for EFSA breakfast cereal µg/kg (contd.)

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.25	0.1	0.1	0.1	0.5	0.1	0.1	0.1
Sample code	Product description	Conc (µg/kg)							
S16-003197	Organic Millet Flakes wholegrain	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003198	Organic Oats	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003199	Organic Express Porridge sachets	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003200	Organic Porridge Chunky Traditional	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003201	Buckwheat toasted muesli	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003202	Organic Granola	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003203	Muesli, puffed spelt, pumpkin, sunflower, flax seeds & sultanas	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003204	Muesli	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003205	Muesli, Quinoa, apple, cranberry & chia seeds	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1

Table 13. Tropane alkaloid results for EFSA breakfast cereal µg/kg (contd.)

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	0.5	0.25	0.5	0.5	0.5	0.05	0.5	0.5
Sample code	Product description	Conc (µg/kg)							
S15-100597	High Bran cereal	<0.5	<0.25	<0.5	<0.5	<0.5	0.38	<0.5	<0.5
S15-100599	Wheat flake biscuits	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100600	Four grain Porridge	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100601	Toasted Muesli with Buckwheat	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100602	Wheat breakfast cereal	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100603	Oatbran flakes Very Berry	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100604	Pumpkin & mango granola	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100605	Wholegrain Wheat bitesize	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100606	Bircher Muesli base	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100607	Maple Sunrise	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100608	Scottish Porridge	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100609	Organic Muesli	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100610	Crispy G/F Buckwheat Flakes Organic	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100611	Buckwheat Puffs G/F Organic	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100613	Wheatflake biscuits Organic	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100614	Sorghum gluten free cereal	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003190	Wheat Bisks	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003191	Original rice and wheat flakes	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003193	Crunchy Spelt Bran Flakes	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003195	Wholegrain Wheat breakfast cereal	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003196	Organic Millet Puffs wholegrain	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5

Table 13. Tropane alkaloid results for EFSA breakfast cereal µg/kg (contd.)

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	0.5	0.25	0.5	0.5	0.5	0.05	0.5	0.5
Sample code	Product description	Conc (µg/kg)							
S16-003197	Organic Millet Flakes wholegrain	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003198	Organic Oats	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003199	Organic Express Porridge sachets	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003200	Organic Porridge Chunky Traditional	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003201	Buckwheat toasted muesli	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003202	Organic Granola	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003203	Muesli, puffed spelt, pumpkin, sunflower, flax seeds & sultanas	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003204	Muesli	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003205	Muesli, Quinoa, apple, cranberry & chia seeds	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5

Table 14. Tropane alkaloid results for EFSA Maize products µg/kg

		Acetyl scopolamine	Anisodamine	Anisodine	Apo atropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.1	0.1	0.1	0.1	0.1	0.05	0.1	0.25
Sample code	Product description	Conc (µg/kg)							
S16-001240	Polenta	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003157	Polenta Paradiso	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003302	Organic Instant Polenta	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003303	Organic Polenta	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003304	Polenta Gluten Free	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003305	Cornmeal Fine	<0.1	<0.1	<0.1	<0.1	<0.1	0.63	<0.1	<0.25
S16-003164	Lightly Salted Tortilla Chips	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100598	Cornflakes	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100612	Organic Cornflakes	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003189	Corn Flakes	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003192	Gluten free Corn Flakes	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003194	Organic Corn Flakes	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003214	Wholegrain Corn Cakes, lightly salted	<0.1	<0.1	<0.1	<0.1	<0.1	0.07	<0.1	<0.25

Table 13. Tropane alkaloid results for EFSA Maize products µg/kg (contd.)

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.25	0.1	0.1	0.1	0.5	0.1	0.1	0.1
Sample code	Product description	Conc (µg/kg)							
S16-001240	Polenta	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003157	Polenta Paradiso	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003302	Organic Instant Polenta	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003303	Organic Polenta	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003304	Polenta Gluten Free	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003305	Cornmeal Fine	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003164	Lightly Salted Tortilla Chips	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100598	Cornflakes	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100612	Organic Cornflakes	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003189	Corn Flakes	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003192	Gluten free Corn Flakes	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003194	Organic Corn Flakes	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003214	Wholegrain Corn Cakes, lightly salted	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1

Table 13. Tropane alkaloid results for EFSA Maize products µg/kg (contd.)

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	0.5	0.25	0.5	0.5	0.5	0.05	0.5	0.5
Sample code	Product description	Conc (µg/kg)							
S16-001240	Polenta	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003157	Polenta Paradiso	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003302	Organic Instant Polenta	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003303	Organic Polenta	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003304	Polenta Gluten Free	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003305	Cornmeal Fine	<0.5	<0.25	<0.5	<0.5	<0.5	0.16	<0.5	<0.5
S16-003164	Lightly Salted Tortilla Chips	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100598	Cornflakes	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100612	Organic Cornflakes	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003189	Corn Flakes	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003192	Gluten free Corn Flakes	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003194	Organic Corn Flakes	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003214	Wholegrain Corn Cakes, lightly salted	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5

Table 15. Tropane Alkaloid results for EFSA bread and pasta µg/kg

		Acetyl scopolamine	Anisodamine	Anisodine	Apo atropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.1	0.1	0.1	0.1	0.1	0.05	0.1	0.25
Sample code	Product description	Conc (µg/kg)							
S15-100615	Rice & Millet Pasta spirals	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100616	Makarony	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100617	Pasta wholemeal	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100618	Deli Sesame Crispbread	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100619	Macaroni	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100620	Linguine	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100621	Fusilli tricolori	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100622	Seeded batch bread	<0.1	<0.1	<0.1	<0.1	<0.1	0.05	<0.1	<0.25
S15-100623	Original Granary Bread	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100624	Multiseed Bread (bakery)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100625	Seeds and Grains Bread	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100626	Brown seeded Loaf	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100627	Brown Bloomer Slices	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100628	Soba Noodles	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100629	Organic Wholewheat fusilli	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100630	Millet Bread	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100631	Seeded sliced Bloomer	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100632	Organic Penne	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100637	Low multigrain Rice cakes	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100646	Buckwheat Crispbread	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100649	Organic Crispbreads	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-001239	Organic Maize & Rice pasta	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003161	Organic Crispbread Classic 3 Seed	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25

Table 15. Tropane Alkaloid results for EFSA bread and pasta µg/kg (contd.)

		Acetyl scopolamine	Anisodamine	Anisodine	Apo atropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.1	0.1	0.1	0.1	0.1	0.05	0.1	0.25
Sample code	Product description	Conc (µg/kg)							
S16-003221	Five seed Flatbreads	<0.1	<0.1	<0.1	<0.1	<0.1	0.07	<0.1	<0.25
S16-003222	Crispbreads Original Rye	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003223	Organic Sunflower seed bread	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003224	Irish Brown Soda Bread	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003225	Multiseed Farmhouse batch loaf	<0.1	<0.1	<0.1	<0.1	<0.1	0.13	<0.1	<0.25
S16-003226	Organic Multiseed Wholemeal	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003227	Naan Bread	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003228	Five Seeds Seeded White Loaf	<0.1	0.13	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003229	Organic Millet Wholegrain Bread	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003230	Seeded Batch Loaf	<0.1	<0.1	<0.1	<0.1	<0.1	0.12	<0.1	<0.25

Table 15. Tropane Alkaloid results for EFSA bread and pasta µg/kg (contd.)

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.25	0.1	0.1	0.1	0.5	0.1	0.1	0.1
Sample code	Product description	Conc (µg/kg)							
S15-100615	Rice & Millet Pasta spirals	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100616	Makarony	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100617	Pasta wholemeal	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100618	Deli Sesame Crispbread	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100619	Macaroni	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100620	Linguine	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100621	Fusilli tricolori	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100622	Seeded batch bread	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100623	Original Granary Bread	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100624	Multiseed Bread (bakery)	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100625	Seeds and Grains Bread	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100626	Brown seeded Loaf	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100627	Brown Bloomer Slices	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100628	Soba Noodles	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100629	Organic Wholewheat fusilli	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100630	Millet Bread	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100631	Seeded sliced Bloomer	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100632	Organic Penne	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100637	Low multigrain Rice cakes	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100646	Buckwheat Crispbread	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100649	Organic Crispbreads	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-001239	Organic Maize & Rice pasta	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1

Table 15. Tropane Alkaloid results for EFSA bread and pasta µg/kg (contd.)

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.25	0.1	0.1	0.1	0.5	0.1	0.1	0.1
Sample code	Product description	Conc (µg/kg)							
S16-003161	Organic Crispbread Classic 3 Seed	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003221	Five seed Flatbreads	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003222	Crispbreads Original Rye	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003223	Organic Sunflower seed bread	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003224	Irish Brown Soda Bread	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003225	Multiseed Farmhouse batch loaf	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003226	Organic Multiseed Wholemeal	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003227	Naan Bread	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003228	Five Seeds Seeded White Loaf	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003229	Organic Millet Wholegrain Bread	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003230	Seeded Batch Loaf	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1

Table 15. Tropane Alkaloid results for EFSA bread and pasta µg/kg (contd.)

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	0.5	0.25	0.5	0.5	0.5	0.05	0.5	0.5
Sample code	Product description	Conc (µg/kg)							
S15-100615	Rice & Millet Pasta spirals	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100616	Makarony	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100617	Pasta wholemeal	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100618	Deli Sesame Crispbread	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100619	Macaroni	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100620	Linguine	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100621	Fusilli tricolori	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100622	Seeded batch bread	<0.5	<0.25	<0.5	<0.5	<0.5	0.05	<0.5	<0.5
S15-100623	Original Granary Bread	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100624	Multiseed Bread (bakery)	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100625	Seeds and Grains Bread	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100626	Brown seeded Loaf	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100627	Brown Bloomer Slices	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100628	Soba Noodles	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100629	Organic Wholewheat fusilli	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100630	Millet Bread	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100631	Seeded sliced Bloomer	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100632	Organic Penne	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100637	Low multigrain Rice cakes	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100646	Buckwheat Crispbread	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100649	Organic Crispbreads	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-001239	Organic Maize & Rice pasta	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5

Table 15. Tropane Alkaloid results for EFSA bread and pasta µg/kg (contd.)

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	0.5	0.25	0.5	0.5	0.5	0.05	0.5	0.5
Sample code	Product description	Conc (µg/kg)							
S16-003161	Organic Crispbread Classic 3 Seed	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003221	Five seed Flatbreads	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003222	Crispbreads Original Rye	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003223	Organic Sunflower seed bread	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003224	Irish Brown Soda Bread	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003225	Multiseed Farmhouse batch loaf	<0.5	<0.25	<0.5	<0.5	<0.5	0.09	<0.5	<0.5
S16-003226	Organic Multiseed Wholemeal	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003227	Naan Bread	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003228	Five Seeds Seeded White Loaf	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003229	Organic Millet Wholegrain Bread	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003230	Seeded Batch Loaf	<0.5	<0.25	<0.5	<0.5	<0.5	0.15	<0.5	<0.5

Table 16. Tropane alkaloid results for EFSA biscuits and baked goods, µg/kg

		Acetyl scopolamine	Anisodamine	Anisodine	Apo atropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.1	0.1	0.1	0.1	0.1	0.05	0.1	0.25
Sample code	Product description	Conc (µg/kg)							
S15-100633	Gluten free Rich Tea biscuit	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100634	Organic Ginger biscuits	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100635	Scottish Shortbread	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100636	Buckwheat Galettes	<0.1	<0.1	<0.1	<0.1	<0.1	1.23	<0.1	<0.25
S15-100638	Madelines	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100639	Seeded crumble mix	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100640	Malted Milk biscuits	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100641	Crackers, sesame, poppy and pumpkin	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100642	Madeira Cake	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100643	Scones	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100644	Fruit Shortcake biscuit	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100645	Digestive biscuits	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100647	Lemon drizzle cake	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100648	Organic Chocolate cake	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100650	Organic Pumpkin and sunflower seed oatcakes	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003163	Breakfast Oaty Breaks	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003187	Crunchy Cereal Bars	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003206	Organic Dark Chocolate chip Cookies	<0.1	<0.1	<0.1	<0.1	<0.1	0.16	<0.1	<0.25
S16-003207	Wholegrain crispbread	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003208	Breakfast biscuits Milk & Cereal	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003209	All butter Maderia Cake	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003210	Madeleines	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25

Table 16. Tropane alkaloid results for EFSA biscuits and baked goods, µg/kg (contd.)

		Acetyl scopolamine	Anisodamine	Anisodine	Apo atropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.1	0.1	0.1	0.1	0.1	0.05	0.1	0.25
Sample code	Product description	Conc (µg/kg)							
S16-003211	Organic All Butter Shortbread Swirls	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003213	Breakfast biscuits Milk & Cereal	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003215	Crackers	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003216	Mini Gingerbread men	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003217	Scottish all butter Shortbread Fingers	<0.1	<0.1	<0.1	<0.1	<0.1	0.14	<0.1	<0.25
S16-003218	Digestive biscuits	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003219	Devon Cream Crackers	<0.1	<0.1	<0.1	<0.1	<0.1	0.05	<0.1	<0.25
S16-003220	Cream Cracker	<0.1	<0.1	<0.1	<0.1	<0.1	0.11	<0.1	<0.25
S16-015533	Super seeded Organic Oatcakes	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-015534	Digestive biscuits	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25

Table 16. Tropane alkaloid results for EFSA biscuits and baked goods, µg/kg (contd.)

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.25	0.1	0.1	0.1	0.5	0.1	0.1	0.1
Sample code	Product description	Conc (µg/kg)							
S15-100633	Gluten free Rich Tea biscuit	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100634	Organic Ginger biscuits	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100635	Scottish Shortbread	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100636	Buckwheat Galettes	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100638	Madelines	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100639	Seeded crumble mix	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100640	Malted Milk biscuits	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100641	Crackers, sesame, poppy and pumpkin	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100642	Madeira Cake	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100643	Scones	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100644	Fruit Shortcake biscuit	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100645	Digestive biscuits	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100647	Lemon drizzle cake	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100648	Organic Chocolate cake	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100650	Organic Pumpkin and sunflower seed oatcakes	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003163	Breakfast Oaty Breaks	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003187	Crunchy Cereal Bars	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003206	Organic Dark Chocolate chip Cookies	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003207	Wholegrain crispbread	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003208	Breakfast biscuits Milk & Cereal	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003209	All butter Maderia Cake	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003210	Madeleines	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1

Table 16. Tropane alkaloid results for EFSA biscuits and baked goods, µg/kg (contd.)

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.25	0.1	0.1	0.1	0.5	0.1	0.1	0.1
Sample code	Product description	Conc (µg/kg)							
S16-003211	Organic All Butter Shortbread Swirls	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003213	Breakfast biscuits Milk & Cereal	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003215	Crackers	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003216	Mini Gingerbread men	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003217	Scottish all butter Shortbread Fingers	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003218	Digestive biscuits	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003219	Devon Cream Crackers	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003220	Cream Cracker	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-015533	Super seeded Organic Oatcakes	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-015534	Digestive biscuits	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1

Table 16. Tropane alkaloid results for EFSA biscuits and baked goods, µg/kg (contd.)

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	0.5	0.25	0.5	0.5	0.5	0.05	0.5	0.5
Sample code	Product description	Conc (µg/kg)							
S15-100633	Gluten free Rich Tea biscuit	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100634	Organic Ginger biscuits	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100635	Scottish Shortbread	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100636	Buckwheat Galettes	<0.5	<0.25	<0.5	<0.5	<0.5	0.65	<0.5	<0.5
S15-100638	Madelines	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100639	Seeded crumble mix	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100640	Malted Milk biscuits	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100641	Crackers, sesame, poppy and pumpkin	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100642	Madeira Cake	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100643	Scones	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100644	Fruit Shortcake biscuit	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100645	Digestive biscuits	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100647	Lemon drizzle cake	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100648	Organic Chocolate cake	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100650	Organic Pumpkin and sunflower seed oatcakes	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003163	Breakfast Oaty Breaks	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003187	Crunchy Cereal Bars	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003206	Organic Dark Chocolate chip Cookies	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003207	Wholegrain crispbread	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003208	Breakfast biscuits Milk & Cereal	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003209	All butter Maderia Cake	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003210	Madeleines	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5

Table 16. Tropane alkaloid results for EFSA biscuits and baked goods, µg/kg (contd.)

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	0.5	0.25	0.5	0.5	0.5	0.05	0.5	0.5
Sample code	Product description	Conc (µg/kg)							
S16-003211	Organic All Butter Shortbread Swirls	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003213	Breakfast biscuits Milk & Cereal	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003215	Crackers	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003216	Mini Gingerbread men	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003217	Scottish all butter Shortbread Fingers	<0.5	<0.25	<0.5	<0.5	<0.5	0.05	<0.5	<0.5
S16-003218	Digestive biscuits	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003219	Devon Cream Crackers	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003220	Cream Cracker	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-015533	Super seeded Organic Oatcakes	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-015534	Digestive biscuits	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5

Table 17. Tropane alkaloid results for EFSA Millet, spelt and other milling products, µg/kg

		Acetyl scopolamine	Anisodamine	Anisodine	Apo atropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.1	0.1	0.1	0.1	0.1	0.05	0.1	0.25
Sample code	Product description	Conc (µg/kg)							
S15-100566	Millet Flour (Bajri Flour)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100567	Sweet White Sorghum Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100568	Millet Grain	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100569	Millet Flour wholegrain stoneground	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100570	Juwar Flour/sorghum flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100571	Bajri Flour/millet	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100572	Sorghum flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100573	Organic Millet Flakes	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100574	Gluten Free Organic Millet Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100575	Golden Millet Flour, Organic	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100576	Organic Millet	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003146	Organic Millet Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003153	Millet Yellow Hulled	<0.2	0.74	<0.1	0.49	0.25	9.79	<0.1	<0.25
S16-003159	Millet Grain	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003309	Millet Flour Wholegrain, stoneground	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-015451	Millet Grain	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-015535	Organic Millet Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100562	Organic Soft Spelt flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003149	Kamut Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003150	Khorasan Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003151	Baking Flour, all purpose	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003306	Beremeal	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003307	Organic Wholemeal Wheat Flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003308	Spelt Flour Organic Wholegrain	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-015550	Organic wholemeal wheat flour	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25

Table 17. Tropane alkaloid results for EFSA Millet, spelt and other milling products, µg/kg (contd.)

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.25	0.1	0.1	0.1	0.5	0.1	0.1	0..1
Sample code	Product description	Conc (µg/kg)							
S15-100566	Millet Flour (Bajri Flour)	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100567	Sweet White Sorghum Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100568	Millet Grain	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100569	Millet Flour wholegrain stoneground	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100570	Juwar Flour/sorghum flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100571	Bajri Flour/millet	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100572	Sorghum flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100573	Organic Millet Flakes	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100574	Gluten Free Organic Millet Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100575	Golden Millet Flour, Organic	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100576	Organic Millet	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003146	Organic Millet Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003153	Millet Yellow Hulled	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	0.12	0.73
S16-003159	Millet Grain	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003309	Millet Flour Wholegrain, stoneground	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-015451	Millet Grain	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-015535	Organic Millet Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100562	Organic Soft Spelt flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003149	Kamut Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003150	Khorasan Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003151	Baking Flour, all purpose	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003306	Beremeal	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003307	Organic Wholemeal Wheat Flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003308	Spelt Flour Organic Wholegrain	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-015550	Organic wholemeal wheat flour	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1

Table 17. Tropane alkaloid results for EFSA Millet, spelt and other milling products, µg/kg (contd.)

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	0.5	0.25	0.5	0.5	0.5	0.05	0.5	0.5
Sample code	Product description	Conc (µg/kg)							
S15-100566	Millet Flour (Bajri Flour)	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100567	Sweet White Sorghum Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100568	Millet Grain	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100569	Millet Flour wholegrain stoneground	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100570	Juwar Flour/sorghum flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100571	Bajri Flour/millet	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100572	Sorghum flour	<0.5	<0.25	<0.5	<0.5	<0.5	1.45	<0.5	<0.5
S15-100573	Organic Millet Flakes	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100574	Gluten Free Organic Millet Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100575	Golden Millet Flour, Organic	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100576	Organic Millet	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003146	Organic Millet Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003153	Millet Yellow Hulled	<0.5	<0.25	0.80	<0.5	4.72	12.9	2.26	0.78
S16-003159	Millet Grain	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003309	Millet Flour Wholegrain, stoneground	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-015451	Millet Grain	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-015535	Organic Millet Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100562	Organic Soft Spelt flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003149	Kamut Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003150	Khorasan Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003151	Baking Flour, all purpose	<0.5	<0.25	2.17	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003306	Beremeal	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003307	Organic Wholemeal Wheat Flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003308	Spelt Flour Organic Wholegrain	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-015550	Organic wholemeal wheat flour	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5

Table 18. Tropane alkaloid results for EFSA infant food samples, µg/kg.

		Acetyl scopolamine	Anisodamine	Anisodine	Apo atropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.1	0.1	0.1	0.1	0.1	0.05	0.1	0.25
Sample code	Product description	Conc / µg/kg							
S15-100577	Baby Rice	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100578	Creamy Oat Porridge	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100579	Banana Museli	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100580	Fruity Crunch Cereal	<0.1	<0.1	<0.1	<0.1	<0.1	3.73	<0.1	<0.25
S15-100581	Rusks	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100582	Raspberry and Banana Muesli	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100583	Multigrain with Mediterranean Vegetables	<0.1	<0.1	<0.1	<0.1	<0.1	0.42	1.30	12.2
S15-100584	Baby Pasta Stars	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100585	Pasta with tomato and spinach	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100586	Carrot cake	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100587	Baby rice with Garden Veg	<0.1	<0.1	<0.1	<0.1	<0.1	0.10	<0.1	<0.25
S15-100588	Gingerbread Men Biscuits	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100589	Organic Baby Pasta	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100590	Multigrain Cereal	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100591	Organic Biscotti	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100592	Banana Porridge	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100593	Organic Plain Biscotti	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100594	Organic Spaghetti Bolognaise	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100595	Organic Creamy Porridge	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S15-100596	Organic Shell pasta tomatoes and courgette	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003162	Kids Animal Cookies	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003165	Fruity Breakfast Porridge	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003166	Creamed Porridge	<0.1	<0.1	<0.1	<0.1	<0.1	1.28	<0.1	<0.25
S16-003167	Organic Apple, Orange & Banana cereal	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25

Table 18. Tropane alkaloid results for EFSA infant food samples, µg/kg (contd.)

		Acetyl scopolamine	Anisodamine	Anisodine	Apo atropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.1	0.1	0.1	0.1	0.1	0.05	0.1	0.25
Sample code	Product description	Conc / µg/kg							
S16-003168	Organic Fruit & Muesli	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003169	Organic Strawberry & Raspberry Muesli	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003170	Banana & Plum Porridge	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003171	Multigrain Fruit Muesli	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003172	Banana & Cinnamon Muesli	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003173	Multigrain Mini Cereal Puffs	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003174	Oats, Apple & Plum Muesli	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003175	Organic Mini Pasta Shells	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003176	Baby Biscuits Apple & Ginger	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003177	Golden Multigrain Biscotti	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003178	Organic Pasta Carbonara	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003179	Organic Vegetables with noodles & chicken	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	0.15	3.57
S16-003180	Multigrain with Cauliflower, Broccoli & Cheese dinner	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003181	Multigrain with Mediterranean Veg dinner	<0.1	<0.1	<0.1	<0.1	<0.1	1.25	1.13	13.4
S16-003182	Blackcurrant Soft Oaty Bars	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003183	Fruity Bakes	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003184	Mini Oat Biscuits	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003185	Spinach Breadsticks	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003186	Salsa Sweetcorn Croc's	<0.1	<0.1	<0.1	<0.1	<0.1	0.45	0.17	8.82
S16-003188	Organic Gluten Free Mini Pasta Stars	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003233	Rusks: Reduced Sugar	<0.1	<0.1	<0.1	<0.1	<0.1	0.10	<0.1	<0.25
S16-003300	Organic Swiss Style Macaroni	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25

Table 18. Tropane alkaloid results for EFSA infant food samples, µg/kg (contd.)

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.25	0.1	0.1	0.1	0.5	0.1	0.1	0.1
Sample code	Product description	Conc / µg/kg							
S15-100577	Baby Rice	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100578	Creamy Oat Porridge	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100579	Banana Museli	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100580	Fruity Crunch Cereal	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100581	Rusks	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100582	Raspberry and Banana Muesli	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100583	Multigrain with Mediterranean Vegetables	<0.25	28.32	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100584	Baby Pasta Stars	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100585	Pasta with tomato and spinach	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100586	Carrot cake	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100587	Baby rice with Garden Veg	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100588	Gingerbread Men Biscuits	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100589	Organic Baby Pasta	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100590	Multigrain Cereal	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100591	Organic Biscotti	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100592	Banana Porridge	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100593	Organic Plain Biscotti	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100594	Organic Spaghetti Bolognaise	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100595	Organic Creamy Porridge	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S15-100596	Organic Shell pasta tomatoes and courgette	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003162	Kids Animal Cookies	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003165	Fruity Breakfast Porridge	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003166	Creamed Porridge	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003167	Organic Apple, Orange & Banana cereal	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1

Table 18. Tropane alkaloid results for EFSA infant food samples, µg/kg (contd.)

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.25	0.1	0.1	0.1	0.5	0.1	0.1	0.1
Sample code	Product description	Conc / µg/kg							
S16-003168	Organic Fruit & Muesli	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003169	Organic Strawberry & Raspberry Muesli	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003170	Banana & Plum Porridge	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003171	Multigrain Fruit Muesli	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003172	Banana & Cinnamon Muesli	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003173	Multigrain Mini Cereal Puffs	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003174	Oats, Apple & Plum Muesli	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003175	Organic Mini Pasta Shells	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003176	aby Biscuits Apple & Ginger	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003177	Golden Multigrain Biscotti	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003178	Organic Pasta Carbonara	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003179	Organic Vegetables with noodles & chicken	<0.25	0.79	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003180	Multigrain with Cauliflower, Broccoli & Cheese dinner	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003181	Multigrain with Mediterranean Veg dinner	<0.25	26.8	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003182	Blackcurrant Soft Oaty Bars	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003183	Fruity Bakes	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003184	Mini Oat Biscuits	<0.25	<0.1	<0.1	<0.1	1.49	<0.1	<0.1	<0.1
S16-003185	Spinach Breadsticks	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003186	Salsa Sweetcorn Croc's	<0.25	12.4	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003188	Organic Gluten Free Mini Pasta Stars	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003233	Rusks: Reduced Sugar	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1
S16-003300	Organic Swiss Style Macaroni Pasta	<0.25	<0.1	<0.1	<0.1	<0.5	<0.1	<0.1	<0.1

Table 18. Tropane alkaloid results for EFSA infant food samples, µg/kg (contd.)

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	0.5	0.25	0.5	0.5	0.5	0.05	0.5	0.5
Sample code	Product description	Conc / µg/kg							
S15-100577	Baby Rice	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100578	Creamy Oat Porridge	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100579	Banana Museli	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100580	Fruity Crunch Cereal	<0.5	<0.25	<0.5	<0.5	<0.5	0.51	<0.5	<0.5
S15-100581	Rusks	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100582	Raspberry and Banana Muesli	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100583	Multigrain with Mediterranean Vegetables	<0.5	<0.25	44.1	<0.5	<0.5	0.13	516	2.13
S15-100584	Baby Pasta Stars	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100585	Pasta with tomato and spinach	<0.5	<0.25	5.97	<0.5	<0.5	<0.05	0.52	<0.5
S15-100586	Carrot cake	<0.5	<0.25	<0.5	<0.5	<0.5	0.18	<0.5	<0.5
S15-100587	Baby rice with Garden Veg	<0.5	<0.25	18.4	<0.5	<0.5	<0.05	0.98	<0.5
S15-100588	Gingerbread Men Biscuits	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100589	Organic Baby Pasta	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100590	Multigrain Cereal	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100591	Organic Biscotti	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100592	Banana Porridge	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100593	Organic Plain Biscotti	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100594	Organic Spaghetti Bolognaise	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100595	Organic Creamy Porridge	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S15-100596	Organic Shell pasta tomatoes and courgette	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003162	Kids Animal Cookies	<0.5	<0.25	<0.5	<0.5	<0.5	0.09	<0.5	<0.5
S16-003165	Fruity Breakfast Porridge	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003166	Creamed Porridge	<0.5	<0.25	<0.5	<0.5	<0.5	0.17	<0.5	<0.5
S16-003167	Organic Apple, Orange & Banana cereal	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5

Table 18. Tropane alkaloid results for EFSA infant food samples, µg/kg (contd.)

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	0.5	0.25	0.5	0.5	0.5	0.05	0.5	0.5
Sample code	Product description	Conc / µg/kg							
S16-003168	Organic Fruit & Muesli	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003169	Organic Strawberry & Raspberry Muesli	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003170	Banana & Plum Porridge	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003171	Multigrain Fruit Muesli	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003172	Banana & Cinnamon Muesli	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003173	Multigrain Mini Cereal Puffs	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003174	Oats, Apple & Plum Muesli	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003175	Organic Mini Pasta Shells	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003176	Baby Biscuits Apple & Ginger	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003177	Golden Multigrain Biscotti	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003178	Organic Pasta Carbonara	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003179	Organic Vegetables with noodles & chicken	<0.5	<0.25	8.67	<0.5	<0.5	<0.05	109	<0.5
S16-003180	Multigrain with Cauliflower, Broccoli & Cheese dinner	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003181	Multigrain with Mediterranean Veg dinner	<0.5	<0.25	41.4	<0.5	<0.5	0.26	773	2.43
S16-003182	Blackcurrant Soft Oaty Bars	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003183	Fruity Bakes	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003184	Mini Oat Biscuits	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003185	Spinach Breadsticks	<0.5	<0.25	<0.5	<0.5	<0.5	0.11	<0.5	<0.5
S16-003186	Salsa Sweetcorn Croc's	<0.5	<0.25	14.7	<0.5	<0.5	0.10	48.2	1.40
S16-003188	Organic Gluten Free Mini Pasta Stars	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003233	Rusks: Reduced Sugar	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003300	Organic Swiss Style Macaroni Pasta	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5

Table 19. Tropane alkaloid results for EFSA Green beans and stir-fry mixes (µg/kg)

		Acetyl scopolamine	Anisodamine	Anisodine	Apo atropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.1	0.1	0.1	0.1	0.1	0.05	0.1	0.25
Sample code	Product description	Conc (µg/kg)							
S16-003257	Sliced Green Beans	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003259	Sliced Green Beans	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003260	Whole Green Beans	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003261	British very fine whole green beans	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003263	Runner Beans	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003256	Vegetable and Beansprout Stir Fry	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003258	Stir Fry Vegetables	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	0.70	6.41
S16-003262	Oriental Cress stir fry	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25
S16-003264	Mixed Vegetable stir fry	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	0.79
S16-006353	Stir fry mix	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.1	<0.25

Table 19. Tropane alkaloid results for EFSA Green beans and stir-fry mixes (µg/kg) (contd.)

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.25	0.25	0.25	0.1	0.5	0.25	0.25	0.25
Sample code	Product description	Conc (µg/kg)							
S16-003257	Sliced Green Beans	<0.25	<0.25	<0.25	<0.1	<0.5	<0.25	<0.25	<0.25
S16-003259	Sliced Green Beans	<0.25	<0.25	<0.25	<0.1	<0.5	<0.25	<0.25	<0.25
S16-003260	Whole Green Beans	<0.25	<0.25	<0.25	<0.1	<0.5	<0.25	<0.25	<0.25
S16-003261	British very fine whole green beans	<0.25	<0.25	<0.25	<0.1	<0.5	<0.25	<0.25	<0.25
S16-003263	Runner Beans	<0.25	<0.25	<0.25	<0.1	<0.5	<0.25	<0.25	<0.25
S16-003256	Vegetable and Beansprout Stir Fry	<0.25	<0.25	<0.25	<0.1	<0.5	<0.25	<0.25	<0.25
S16-003258	Stir Fry Vegetables	<0.25	11.6	<0.25	<0.1	<0.5	<0.25	<0.25	<0.25
S16-003262	Oriental Cress stir fry	<0.25	<0.25	<0.25	<0.1	<0.5	<0.25	<0.25	<0.25
S16-003264	Mixed Vegetable stir fry	<0.25	0.64	<0.25	<0.1	<0.5	<0.25	<0.25	<0.25
S16-006353	Stir fry mix	<0.25	<0.25	<0.25	<0.1	<0.5	<0.25	<0.25	<0.25

Table 19. Tropane alkaloid results for EFSA Green beans and stir-fry mixes (µg/kg)

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	0.5	0.25	0.5	0.5	0.5	0.05	0.5	0.5
Sample code	Product description	Conc (µg/kg)							
S16-003257	Sliced Green Beans	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003259	Sliced Green Beans	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003260	Whole Green Beans	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003261	British very fine whole green beans	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003263	Runner Beans	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003256	Vegetable and Beansprout Stir Fry	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5
S16-003258	Stir Fry Vegetables	<0.5	<0.25	43.6	<0.5	<0.5	<0.05	571	1.05
S16-003262	Oriental Cress stir fry	<0.5	<0.25	15.1	<0.5	<0.5	<0.05	24.1	<0.5
S16-003264	Mixed Vegetable stir fry	<0.5	<0.25	19.5	<0.5	<0.5	<0.05	27.9	<0.5
S16-006353	Stir fry mix	<0.5	<0.25	<0.5	<0.5	<0.5	<0.05	<0.5	<0.5

Table 20. Tropane alkaloid results for EFSA Herbal teas (dry weight basis), µg/kg

		Acetyl scopolamine	Anisodamine	Anisodine	Apo atropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.25	0.25	0.25	0.25	0.25	0.1	0.5	0.5
Sample code	Product description	Conc / µg/kg							
S16-003154	Nettle Tea	<0.25	<0.25	<0.25	<0.25	<0.25	<0.1	<0.5	<0.5
S16-003155	Redbush teabags	<0.25	<0.25	<0.25	<0.25	<0.25	<0.1	<0.5	<0.5
S16-003156	Pure Peppermint tea	<0.25	<0.25	<0.25	<0.25	<0.25	0.95	<0.5	<0.5
S16-003231	Camomile tea	<0.25	<0.25	<0.25	<0.25	<0.25	0.84	<0.5	<0.5
S16-003238	Peppermint Infusion Caffeine Free Tea bags	<0.25	<0.25	<0.25	<0.25	<0.25	1.54	<0.5	<0.5
S16-003242	Nettle Teabags	<0.25	<0.25	<0.25	<0.25	<0.25	2.26	<0.5	<0.5
S16-003243	Peppermint Teabags	<0.25	3.33	<0.25	0.32	0.28	50.2	<0.5	<0.5
S16-003244	Camomile herbal infusion	<0.25	<0.25	<0.25	<0.25	<0.25	<0.1	<0.5	<0.5
S16-003246	Peppermint tea	<0.25	2.48	<0.25	0.14	<0.25	129	<0.5	<0.5
S16-003247	Redbush Naturally caffeine free	<0.25	<0.25	<0.25	<0.25	<0.25	<0.1	<0.5	<0.5
S16-003250	Rooibos tea bag	<0.25	<0.25	<0.25	<0.25	<0.25	<0.1	<0.5	<0.5
S16-003251	Rooibosch Tea	<0.25	<0.25	<0.25	<0.25	<0.25	2.1	<0.5	<0.5
S16-003252	Organic Lemon Balm Herb Tea	<0.25	<0.25	<0.25	<0.25	<0.25	<0.1	<0.5	<0.5
S16-003253	Nettle Tea	<0.25	<0.25	<0.25	<0.25	<0.25	<0.1	<0.5	<0.5
S16-003254	Peppermint Tea	<0.25	<0.25	<0.25	<0.25	<0.25	0.38	<0.5	<0.5
S16-003255	Camomile Tea	<0.25	<0.25	<0.25	<0.25	<0.25	2.44	<0.5	<0.5
S16-003310	Cool mint green Tea	<0.25	<0.25	<0.25	<0.25	<0.25	<0.1	<0.5	<0.5
S16-003311	Rooibos Tea	<0.25	<0.25	<0.25	<0.25	<0.25	1.55	<0.5	<0.5
S16-003312	Camomile tea	<0.25	<0.25	<0.25	<0.25	<0.25	<0.1	<0.5	<0.5
S16-015464	Chamomile with Lemon balm and manuka honey	<0.25	<0.25	<0.25	<0.25	<0.25	<0.1	<0.5	0.78

Table 20. Tropane alkaloid results for EFSA Herbal teas (dry weight basis), µg/kg (contd.)

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.5	0.5	0.25	0.25	1.0	0.25	0.25	0.25
Sample code	Product description	Conc / µg/kg							
S16-003154	Nettle Tea	<0.5	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25
S16-003155	Redbush teabags	<0.5	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25
S16-003156	Pure Peppermint tea	<0.5	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25
S16-003231	Camomile tea	<0.5	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25
S16-003238	Peppermint Infusion Caffeine Free Tea bags	<0.5	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25
S16-003242	Nettle Teabags	<0.5	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25
S16-003243	Peppermint Teabags	<0.5	<0.5	<0.25	<0.25	<1	<0.25	0.41	0.98
S16-003244	Camomile herbal infusion	<0.5	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25
S16-003246	Peppermint tea	<0.5	<0.5	<0.25	<0.25	<1	<0.25	0.43	0.3
S16-003247	Redbush Naturally caffeine free	<0.5	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25
S16-003250	Rooibos tea bag	<0.5	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25
S16-003251	Rooibosch Tea	<0.5	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25
S16-003252	Organic Lemon Balm Herb Tea	<0.5	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25
S16-003253	Nettle Tea	<0.5	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25
S16-003254	Peppermint Tea	<0.5	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25
S16-003255	Camomile Tea	<0.5	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25
S16-003310	Cool mint green Tea	<0.5	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25
S16-003311	Rooibos Tea	<0.5	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25
S16-003312	Camomile tea	<0.5	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25
S16-015464	Chamomile with Lemon balm and manuka honey	19.1	<0.5	<0.25	<0.25	<1	<0.25	<0.25	<0.25

Table 20. Tropane alkaloid results for EFSA Herbal teas (dry weight basis), µg/kg (contd.)

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	2.5	0.25	2.5	1.0	1.0	0.1	2.5	1.0
Sample code	Product description	Conc / µg/kg							
S16-003154	Nettle Tea	<2.5	<0.25	5.49	<1	<1	<0.1	<2.5	<1
S16-003155	Redbush teabags	<2.5	<0.25	<2.5	<1	<1	<0.1	<2.5	<1
S16-003156	Pure Peppermint tea	<2.5	<0.25	9.26	<1	<1	1.35	<2.5	<1
S16-003231	Camomile tea	<2.5	<0.25	<2.5	<1	<1	<0.1	<2.5	<1
S16-003238	Peppermint Infusion Caffeine Free Tea bags	<2.5	<0.25	<2.5	<1	<1	<0.1	12.5	<1
S16-003242	Nettle Teabags	<2.5	<0.25	<2.5	<1	<1	0.74	14.0	<1
S16-003243	Peppermint Teabags	<2.5	<0.25	<2.5	<1	<1	34.1	3.43	<1
S16-003244	Camomile herbal infusion	<2.5	<0.25	<2.5	<1	<1	0.17	<2.5	2.78
S16-003246	Peppermint tea	<2.5	<0.25	<2.5	<1	<1	33.7	9.62	<1
S16-003247	Redbush Naturally caffeine free	<2.5	<0.25	<2.5	<1	<1	<0.1	<2.5	<1
S16-003250	Rooibos tea bag	<2.5	<0.25	<2.5	<1	<1	<0.1	<2.5	<1
S16-003251	Rooibosch Tea	<2.5	<0.25	<2.5	<1	<1	0.37	<2.5	<1
S16-003252	Organic Lemon Balm Herb Tea	<2.5	<0.25	<2.5	<1	<1	<0.1	2.82	<1
S16-003253	Nettle Tea	<2.5	<0.25	3.29	<1	<1	<0.1	<2.5	<1
S16-003254	Peppermint Tea	<2.5	<0.25	<2.5	<1	<1	1.02	<2.5	<1
S16-003255	Camomile Tea	<2.5	<0.25	<2.5	<1	<1	<0.1	3.97	<1
S16-003310	Cool mint green Tea	<2.5	<0.25	<2.5	<1	<1	<0.1	<2.5	<1
S16-003311	Rooibos Tea	<2.5	<0.25	<2.5	<1	<1	0.33	<2.5	<1
S16-003312	Camomile tea	<2.5	<0.25	<2.5	<1	<1	<0.1	<2.5	2.96
S16-015464	Chamomile with Lemon balm and manuka honey	<2.5	<0.25	<2.5	<1	<1	<0.1	9.27	<1

Table 21. Calystegine results for EFSA sampled potatoes, whole product basis, mg/kg.

		Calystegine A3	Calystegine A5	Calystegine B1	Calystegine B2	Calystegine B3	Calystegine B4
	Reporting limit (assigned LOD)	0.5	1.0	0.25	0.25	0.25	0.25
Sample code	Product description	Conc / mg/kg					
S15-100667	Marabel Potatoes	118.3	<1.0	<0.25	51.9	<0.25	4.1
S15-100668	Herby Wedges	211.4	1.2	<0.25	127.1	<0.25	6.9
S15-100669	Baby Potatoes	101.1	<1.0	<0.25	13.1	<0.25	7.6
S15-100670	Scottish Charlotte Potatoes	167.8	<1.0	<0.25	107.2	<0.25	5.3
S15-100671	Jazzy Small Potato	174.0	<1.0	<0.25	72.7	<0.25	4.1
S15-100672	Jacket Potatoes	151.5	<1.0	<0.25	77.0	<0.25	3.7
S15-100673	Baby Potatoes UK	201.3	<1.0	<0.25	60.9	<0.25	6.7
S15-100674	All rounder potatoes	96.6	<1.0	<0.25	33.3	<0.25	2.4
S15-100675	King Edward Potatoes	149.7	<1.0	<0.25	40.7	<0.25	1.3
S15-100676	Rooster potatoes	99.6	<1.0	<0.25	39.3	<0.25	3.1
S15-100677	Potatoes	193.7	<1.0	<0.25	73.7	<0.25	0.5
S15-100678	Anya potatoes	69.9	<1.0	<0.25	26.0	<0.25	2.2
S15-100679	British Charlotte potatoes	205.9	<1.0	<0.25	122.7	<0.25	5.5
S15-100680	Baking Potatoes	51.8	<1.0	<0.25	15.9	<0.25	8.1
S15-100681	ELFE potato	67.4	<1.0	<0.25	20.2	<0.25	2.2
S15-100682	Corolle potatoes	129.3	<1.0	<0.25	49.2	<0.25	0.7
S15-100683	Ruby Gem Potatoes	261.5	<1.0	<0.25	90.2	<0.25	11.2
S15-100684	Loose potatoes	142.7	10.6	<0.25	80.2	<0.25	0.6
S15-100685	Organic Potatoes	262.2	<1.0	<0.25	47.3	<0.25	4.2
S15-100686	Organic Salad Potatoes	131.9	<1.0	<0.25	67.6	<0.25	4.5
S15-100687	Organic Lady Balfour baking potatoes	309.9	<1.0	<0.25	56.7	<0.25	4.4
S15-100688	Organic Baby Salad potatoes	35.1	<1.0	<0.25	10.5	<0.25	1.6
S15-100689	Baby Organic Potatoes	13.6	<1.0	<0.25	16.9	<0.25	1.5
S16-003265	Charlotte Potatoes	236.2	4.1	<0.25	126.4	<0.25	13.5
S16-003266	Charlotte Potatoes	110.9	5.4	<0.25	105.5	<0.25	4.3
S16-003267	British Baby Potatoes	23.7	<1.0	<0.25	14.9	<0.25	2.4

Table 21. Calystegine results for EFSA sampled potatoes, whole product basis, mg/kg (contd.)

		Calystegine A3	Calystegine A5	Calystegine B1	Calystegine B2	Calystegine B3	Calystegine B4
	Reporting limit (assigned LOD)	0.5	1.0	0.25	0.25	0.25	0.25
Sample code	Product description	Conc / mg/kg					
S16-003268	Baking potatoes	23.9	<1.0	<0.25	5.9	<0.25	0.7
S16-003269	Baby potatoes	18.1	<1.0	<0.25	4.1	<0.25	1.2
S16-003270	Organic baby potatoes	112.9	6.3	<0.25	109.3	<0.25	4.7
S16-003271	Jersey Royals	90.9	<1.0	<0.25	24.7	<0.25	0.8
S16-003272	Baby Potatoes	101.3	<1.0	<0.25	23.7	<0.25	2.4
S16-003273	Miniature Potatoes	31.5	<1.0	<0.25	17.5	<0.25	0.5
S16-003274	Organic Baby Potatoes	159.5	<1.0	<0.25	51.1	<0.25	16.7
S16-003275	Potatoes	134.4	<1.0	<0.25	39.5	<0.25	4.0
S16-003276	Organic White Potato	132.7	<1.0	<0.25	18.6	<0.25	1.7
S16-003277	Organic White Potatoes	77.1	<1.0	<0.25	45.8	<0.25	2.6
S16-003278	Organic Baby Potatoes	84.2	<1.0	<0.25	27.3	<0.25	0.8
S16-003279	British White Potatoes	33.4	<1.0	<0.25	7.4	<0.25	1.6
S16-003280	British Baby Potatoes	63.0	2.4	<0.25	21.5	<0.25	4.8
S16-003281	Rooster Potatoes	96.2	<1.0	<0.25	38.2	<0.25	4.0
S16-003282	Mini Potatoes	179.6	<1.0	<0.25	69.0	<0.25	5.6
S16-003283	Baby Maris Peer potato	13.0	<1.0	<0.25	5.4	<0.25	1.1
S16-003284	Ruby Gem Potatoes	221.6	<1.0	<0.25	71.7	<0.25	15.8
S16-003285	Miniature potatoes	75.1	<1.0	<0.25	24.0	<0.25	1.2

Table 22. Calystegine results for EFSA sampled aubergines, whole product basis, mg/kg

		Calystegine A3	Calystegine A5	Calystegine B1	Calystegine B2	Calystegine B3	Calystegine B4
	Reporting limit (assigned LOD)	0.5	1.0	0.25	0.25	0.25	0.25
Sample code	Product description	Conc / mg/kg					
S15-100690	Aubergines	5.8	<1.0	7.1	22.4	<0.25	<0.25
S15-100691	Aubergines	6.1	<1.0	6.6	18.8	<0.25	<0.25
S15-100692	Aubergines	3.6	<1.0	4.8	20.5	<0.25	<0.25
S15-100693	Aubergine	5.9	<1.0	6.2	30.5	<0.25	<0.25
S15-100694	Aubergines	8.3	<1.0	8.1	40.6	<0.25	<0.25
S15-100695	Aubergines Organic	6.1	<1.0	6.8	33.2	<0.25	<0.25
S15-100696	Aubergines	7.5	<1.0	5.8	29.6	<0.25	<0.25
S15-100697	Aubergines	0.7	<1.0	7.4	4.7	<0.25	<0.25
S16-003232	Aubergines	1.1	<1.0	0.9	1.5	<0.25	<0.25
S16-003286	Aubergines	<0.5	<1.0	0.5	0.7	<0.25	<0.25
S16-003287	Aubergines	1.1	<1.0	2.8	11.6	<0.25	<0.25
S16-003288	Aubergines	3.8	<1.0	1.9	3.5	<0.25	<0.25
S16-003289	Aubergine	1.6	<1.0	3.4	14.4	<0.25	<0.25
S16-003290	Aubergine	2.1	<1.0	1.2	1.9	<0.25	<0.25
S16-003315	Baby Aubergines	28.1	<1.0	28.6	124.8	<0.25	<0.25

Table 23. Tropane alkaloid results for additional UK flour and maize product samples, (as purchased) in µg/kg.

		Acetyl scopolamine	Anisodamine	Anisodine	Apo atropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.1	0.1	0.1	0.1	0.1	0.05	0.2*	0.25
Sample code	Product description	Conc (µg/kg)							
S15-100708	Gluten Free Lasagne	< 0.1	< 0.1	< 0.1	< 0.15*	< 0.1	0.05	< 0.2	< 0.25
S15-100709	Ready Made Polenta	< 0.1	< 0.1	< 0.1	< 0.22*	< 0.17*	0.22	< 0.2	< 0.25
S15-100710	Polenta 100% pre-cooked maize flour	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.06*	< 0.2	< 0.25
S15-100711	Cornflour	< 0.1	< 0.1	< 0.1	< 0.17*	< 0.17*	< 0.06*	< 0.2	< 0.25
S15-100712	Cornmeal Coarse	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.06*	< 0.2	< 0.25
S16-003301	Maizemeal Medium Polenta	< 0.1	< 0.1	< 0.1	< 0.1	< 0.01	< 0.06*	< 0.2	< 0.25
S15-100713	00 Flour	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.08*	< 0.2	< 0.25
S15-100714	Rye Flour	< 0.1	< 0.15*	< 0.1	< 0.19*	< 0.22*	< 0.08*	< 0.2	< 0.25
S15-100715	Self Raising Flour	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.09*	< 0.2	< 0.25
S15-100716	Spelt Flour Organic Wholegrain	< 0.1	< 0.1	< 0.1	< 0.16*	< 0.2*	< 0.09*	< 0.2	< 0.25
S15-100717	Organic Wholegrain Spelt Flour	< 0.1	< 0.1	< 0.1	< 0.19*	< 0.24*	< 0.08*	< 0.2	< 0.25

* Reporting limit raised due to interferences from high background signal or low recovery

(-) results in parenthesis were measured values but below the reporting limit

Table 23. Tropane alkaloid results for additional UK flour and maize product samples, (as purchased) in µg/kg (contd.)

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.25	0.1	0.1	0.1	0.5	0.1	0.1	0.1
Sample code	Product description	Conc (µg/kg)							
S15-100708	Gluten Free Lasagne	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S15-100709	Ready Made Polenta	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S15-100710	Polenta 100% pre-cooked maize flour	< 0.25	< 0.01	< 0.1	< 0.1	< 0.	< 0.1	< 0.1	< 0.1
S15-100711	Cornflour	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S15-100712	Cornmeal Coarse	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-003301	Maizemeal Medium Polenta	< 0.25	< 0.1	< 0.18*	< 0.1	< 0.5	< 0.1	< 0.19*	< 0.1
S15-100713	00 Flour	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S15-100714	Rye Flour	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S15-100715	Self Raising Flour	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S15-100716	Spelt Flour Organic Wholegrain	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S15-100717	Organic Wholegrain Spelt Flour	< 0.25	< 0.1	< 0.1	< 0.1	< 0.	< 0.1	< 0.1	< 0.1

* Reporting limit raised due to interferences from high background signal or low recovery

(-) results in parenthesis were measured values but below the reporting limit

Table 23. Tropane alkaloid results for additional UK flour and maize product samples, (as purchased) in µg/kg (contd.)

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	0.5	0.25	0.5	0.5	0.5	0.05	0.5	0.5
Sample code	Product description	Conc (µg/kg)							
S15-100708	Gluten Free Lasagne	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.06*	< 0.5	< 1.3*
S15-100709	Ready Made Polenta	< 0.61*	< 0.25	< 0.5	< 0.5	< 0.5	0.06	< 0.5	< 0.5
S15-100710	Polenta 100% pre-cooked maize flour	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.08*	< 0.5	< 0.5
S15-100711	Cornflour	< 0.86*	< 0.25	< 0.5	< 0.5	< 0.55*	< 0.08*	< 0.5	< 0.72*
S15-100712	Cornmeal Coarse	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.08*	< 0.5	< 0.5
S16-003301	Maizemeal Medium Polenta	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.06*	< 0.5	< 0.5
S15-100713	00 Flour	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1*	< 0.5	< 0.5
S15-100714	Rye Flour	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.09*	< 0.5	< 0.5
S15-100715	Self Raising Flour	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1*	< 0.5	< 0.5
S15-100716	Spelt Flour Organic Wholegrain	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.11*	< 0.5	< 0.5
S15-100717	Organic Wholegrain Spelt Flour	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.11*	< 0.5	< 0.5

* Reporting limit raised due to interferences from high background signal or low recovery

(-) results in parenthesis were measured values but below the reporting limit

Table 24. Tropane alkaloid results for flour samples supplied by UK millers (nabim), µg/kg

		Acetyl scopolamine	Anisodamine	Anisodine	Apo atropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.1	0.1	0.1	0.1	0.1	0.1*	0.1	0.25
Sample code	Product description	Conc (µg/kg)							
S16-015548	Buckwheat Wholemeal Flour	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.33*
S16-015549	Millet Wholemeal Flour	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S16-015550	Cotswold Premium Organic Wholemeal flour	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S16-015551	Chapatti Wholemeal B GRW	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S16-015558	Wholemeal flour	< 0.21*	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S16-015559	Wholemeal flour TL 960	< 0.14*	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S16-015560	Wholemeal Flour	< 0.18*	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S16-015561	Stoneground Organic Premium Wholemeal - STOPR	< 0.17*	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S16-015562	Wholemeal A GRW	< 0.23*	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S16-015563	Wholemeal Flour	< 0.17*	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S16-015564	Biscuit Wholemeal	< 0.2*	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S16-015565	Fine Plain English Wholemeal Flour	< 0.15*	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S16-015566	Number 2 Chapatti Wholemeal Flour	< 0.19*	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S16-015567	Wholemeal Wheat flour	< 0.24*	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S16-015568	Wholemeal flour	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S16-015569	Wholemeal Plain Flour	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S16-015570	Windsor Wholemeal Flour	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.65*

* Reporting limit raised due to interferences from high background signal or low recovery

(-) results in parenthesis were measured values but below the reporting limit

Table 24. Tropane alkaloid results for flour samples supplied by UK millers (nabim), µg/kg (contd.)

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.3*	0.1	0.1	0.1	0.5	0.1	0.1	0.1
Sample code	Product description	Conc (µg/kg)							
S16-015548	Buckwheat Wholemeal Flour	< 0.3	< 0.19*	< 0.1	< 0.1	< 0.74*	< 0.1	< 0.1	< 0.1
S16-015549	Millet Wholemeal Flour	< 0.3	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-015550	Cotswold Premium Organic Wholemeal flour	< 0.3	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-015551	Chapatti Wholemeal B GRW	< 0.3	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-015558	Wholemeal flour	< 0.3	< 0.1	< 0.18*	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-015559	Wholemeal flour TL 960	< 0.3	< 0.15*	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-015560	Wholemeal Flour	< 0.3	< 0.1	< 0.17*	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-015561	Stoneground Organic Premium Wholemeal - STOPR	< 0.3	< 0.1	< 0.17*	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-015562	Wholemeal A GRW	< 0.3	< 0.1	< 0.15*	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-015563	Wholemeal Flour	< 0.3	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-015564	Biscuit Wholemeal	< 0.3	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-015565	Fine Plain English Wholemeal Flour	< 0.3	< 0.1	< 0.17*	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-015566	Number 2 Chapatti Wholemeal Flour	< 0.3	< 0.1	< 0.15*	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-015567	Wholemeal Wheat flour	< 0.3	< 0.15*	< 0.16*	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-015568	Wholemeal flour	< 0.3	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-015569	Wholemeal Plain Flour	< 0.3	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-015570	Windsor Wholemeal Flour	<0.3	<0.1	< 0.1	< 0.2*	<0.5	<0.1	<0.1	< 0.45*

* Reporting limit raised due to interferences from high background signal or low recovery

(-) results in parenthesis were measured values but below the reporting limit

Table 24. Tropane alkaloid results for flour samples supplied by UK millers (nabim), µg/kg (contd.)

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	0.5	0.25	0.5	0.5	0.5	0.1*	0.5	0.5
Sample code	Product description	Conc (µg/kg)							
S16-015548	Buckwheat Wholemeal Flour	< 6.67*	< 0.25	< 0.65*	< 0.83*	< 1.75*	< 0.1	< 0.5	< 0.65*
S16-015549	Millet Wholemeal Flour	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.85*
S16-015550	Cotswold Premium Organic Wholemeal flour	< 0.67*	< 0.25	< 1.3*1	< 0.5	< 0.74*	< 0.15*	< 0.5	< 0.67*
S16-015551	Chapatti Wholemeal B GRW	< 0.59*	< 0.25	< 2.51*	< 0.5	< 0.75*	< 0.1	< 0.5	< 0.63*
S16-015558	Wholemeal flour	< 0.63*	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.64*
S16-015559	Wholemeal flour TL 960	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.5
S16-015560	Wholemeal Flour	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.62*
S16-015561	Stoneground Organic Premium Wholemeal - STOPR	< 0.7*	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.5
S16-015562	Wholemeal A GRW	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.5
S16-015563	Wholemeal Flour	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.22*	< 0.5	< 0.55*
S16-015564	Biscuit Wholemeal	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.68*
S16-015565	Fine Plain English Wholemeal Flour	< 0.61*	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.5
S16-015566	Number 2 Chapatti Wholemeal Flour	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.5
S16-015567	Wholemeal Wheat flour	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.69*
S16-015568	Wholemeal flour	< 0.69*	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.68*
S16-015569	Wholemeal Plain Flour	< 0.73*	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.66*
S16-015570	Windsor Wholemeal Flour	<0.65*	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.5

* Reporting limit raised due to interferences from high background signal or low recovery

(-) results in parenthesis were measured values but below the reporting limit

Table 25. Tropane alkaloid results for additional UK infant food samples, (as purchased) in µg/kg.

		Acetyl scopolamine	Anisodamine	Anisodine	Apo atropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.1	0.1	0.1	0.1	0.1	0.05	0.1	0.25
Sample code	Product description	Conc (µg/kg)							
S15-100698	Fruit & nut mix muesli with oats & cereal 10m+	< 0.1	< 0.1	< 0.1	< 0.15*	< 0.1	< 0.06*	< 0.18*	< 0.49*
S15-100699	Apricot and Peach Muesli	< 0.1	< 0.1	< 0.1	< 0.15*	< 0.1	< 0.05	< 0.19*	< 0.25
S15-100700	Multigrain Cereal 7+ months	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.07*	< 0.1	< 0.25
S15-100701	Organic 3-Grain Porridge	< 0.1	< 0.1	< 0.1	< 0.15*	< 0.1	< 0.05	< 0.17*	< 0.25
S15-100702	Multigrain with Mediterranean Vegetables	< 0.1	< 0.1	< 0.1	< 0.16*	< 0.1	1.14	1.03	8.84
S15-100703	Millet Organic Porridge	< 0.1	< 0.1	< 0.1	< 0.17*	< 0.1	0.16	< 0.18*	< 0.25
S15-100704	Organic Spelt Porridge	< 0.1	< 0.1	< 0.1	< 0.25*	< 0.1	< 0.05	< 0.23*	< 0.25
S15-100705	Moroccan Chicken Tagine	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.06*	< 0.1	< 0.25
S15-100706	Curried Chicken Dhal	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.06*	< 0.1	< 0.25
S15-100707	Tomato & Chunky chickpea curry	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.06*	< 0.1	<0.25 (0.16)
S16-001241	Freshly Milled Super Fine lentil and chick pea flour	< 0.1	< 0.1	< 0.1	< 0.1	< 0.15*	< 0.06*	< 0.1	< 0.25
S16-003291	Summer Fruit Multigrain cereal	< 0.15*	< 0.1	< 0.1	< 0.1	< 0.1	< 0.16*	< 0.1	< 0.31*
S16-003292	Thai Curry with Noodles and Coconut	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	<0.05 (0.03)	0.17	3.39
S16-003293	Banana Porridge	< 0.1	< 0.1	< 0.1	< 0.17*	< 0.1	0.07	< 0.18*	< 0.25
S16-003294	Multigrain with Carrot, Sweetcorn & Cheese	< 0.1	< 0.1	< 0.1	< 0.1	<0.1 (0.08)	0.05	< 0.1	< 0.88*
S16-003295	Multigrain Banana Porridge	< 0.1	< 0.1	< 0.1	< 0.16*	< 0.1	0.05	< 0.19*	< 0.47*
S16-003296	Summer Fruit Multigrain cereal	< 0.1	< 0.1	< 0.1	< 0.17*	< 0.1	0.05	< 0.19*	< 0.25
S16-003297	First Muesli	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.05	< 0.1	< 0.25
S16-003298	Strawberry, Raspberry & Blueberry Muesli	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.05	< 0.1	< 0.25
S16-003299	Gram Flour	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.05	< 0.1	< 0.25

* Reporting limit raised due to interferences from high background signal or low recovery

(-) results in parenthesis were measured values but below the reporting limit

Table 25. Tropane alkaloid results for additional UK infant food samples, (as purchased) in µg/kg (contd.)

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.25	0.1	0.1	0.1	0.5	0.1	0.1	0.1
Sample code	Product description	Conc (µg/kg)							
S15-100698	Fruit & nut mix muesli with oats & cereal 10m+	< 0.25	< 0.24*	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 2.15*
S15-100699	Apricot and Peach Muesli	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.23*
S15-100700	Multigrain Cereal 7+ months	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S15-100701	Organic 3-Grain Porridge	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S15-100702	Multigrain with Mediterranean Vegetables	< 0.25	38.6	< 0.1	< 0.1	< 0.5	< 0.1	< 0.25*	< 0.1
S15-100703	Millet Organic Porridge	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S15-100704	Organic Spelt Porridge	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S15-100705	Moroccan Chicken Tagine	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S15-100706	Curried Chicken Dhal	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S15-100707	Tomato & Chunky chickpea curry	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-001241	Freshly Milled Super Fine lentil and chick pea flour	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-003291	Summer Fruit Multigrain cereal	< 0.25	< 0.17*	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.28*
S16-003292	Thai Curry with Noodles and Coconut	< 0.25	3.28	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-003293	Banana Porridge	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.75*
S16-003294	Multigrain with Carrot, Sweetcorn & Cheese	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	0.12
S16-003295	Multigrain Banana Porridge	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 7.99*
S16-003296	Summer Fruit Multigrain cereal	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.72*
S16-003297	First Muesli	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-003298	Strawberry, Raspberry & Blueberry Muesli	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-003299	Gram Flour	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1

* Reporting limit raised due to interferences from high background signal or low recovery

(-) results in parenthesis were measured values but below the reporting limit

Table 25. Tropane alkaloid results for additional UK infant food samples, (as purchased) in µg/kg (contd.)

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	0.5	0.25	0.5	0.5	0.5	0.1	0.5	0.5
Sample code	Product description	Conc (µg/kg)							
S15-100698	Fruit & nut mix muesli with oats & cereal 10m+	< 0.55*	< 0.25	< 0.5	< 0.5	< 0.5	< 0.23*	< 0.5	< 0.21
S15-100699	Apricot and Peach Muesli	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.5
S15-100700	Multigrain Cereal 7+ months	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.5
S15-100701	Organic 3-Grain Porridge	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.5
S15-100702	Multigrain with Mediterranean Vegetables	< 0.5	< 0.25	25.8	< 0.5	< 0.5	0.26	492	1.66
S15-100703	Millet Organic Porridge	< 0.56*	< 0.25	< 0.5	< 0.5	< 0.55*	0.1	< 0.5	< 0.67*
S15-100704	Organic Spelt Porridge	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.62*
S15-100705	Moroccan Chicken Tagine	1.64	< 0.25	8.31	< 0.5	< 0.5	< 0.1	1.31	< 0.5
S15-100706	Curried Chicken Dhal	< 0.59*	< 0.25	12.8	< 0.5	< 0.5	< 0.1	< 0.5	< 0.5
S15-100707	Tomato & Chunky chickpea curry	4.53	< 0.25	29.6	< 0.5	< 0.5	< 0.1	2.51	<1.5 (0.37)
S16-001241	Freshly Milled Super Fine lentil and chick pea flour	< 0.5	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.5
S16-003291	Summer Fruit Multigrain cereal	< 0.73*	< 0.25	< 0.5	< 0.5	< 0.78*	< 0.19*	< 0.5	< 0.57*
S16-003292	Thai Curry with Noodles and Coconut	< 0.63*	< 0.25	5.51	< 0.5	< 0.5	< 0.1	97.8	0.60
S16-003293	Banana Porridge	< 0.5	< 0.25	< 0.5	< 0.5	< 0.61*	< 0.1	< 2*	< 0.56*
S16-003294	Multigrain with Carrot, Sweetcorn & Cheese	< 0.5	< 0.25	< 0.67*	< 0.5	< 0.5	0.88	< 0.5	< 0.5
S16-003295	Multigrain Banana Porridge	< 0.71*	< 0.25	< 0.5	< 0.5	< 0.5	< 0.1	< 0.5	< 0.5
S16-003296	Summer Fruit Multigrain cereal	< 0.5	< 0.25	< 0.5	< 0.5	< 0.61*	< 0.1	< 1.39*	< 0.59*
S16-003297	First Muesli	< 0.59*	< 0.25	< 0.98*	< 0.5	< 0.59*	< 0.1	< 0.64*	< 0.5
S16-003298	Strawberry, Raspberry & Blueberry Muesli	< 0.5	< 0.25	< 0.91*	< 0.5	< 0.59*	< 0.1	< 0.61*	< 0.5
S16-003299	Gram Flour	< 0.5	< 0.25	< 1.05*	< 0.68*	< 0.94*	< 0.1 (0.03)	< 0.5	< 1.04*

* Reporting limit raised due to interferences from high background signal or low recovery

(-) results in parenthesis were measured values but below the reporting limit

Table 26. Tropane alkaloid results for additional UK oilseed samples, in µg/kg.

		Acetyl scopolamine	Anisodamine	Anisodine	Apo atropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.1	0.1	0.1	0.1	0.1	0.1*	0.1	0.25
Sample code	Product description	Conc (µg/kg)							
S16-003313	Milled Linseed	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S16-003314	Golden Flaxseed Cold Milled Organic	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S16-003316	Sunflower Seed Kernels	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25
S15-100720	Brown Linseed	< 0.1	< 0.1	< 0.1	< 0.36*	< 0.19*	< 0.1	< 0.15*	< 0.25
S15-100718	Golden Linseeds	< 0.1	< 0.1	< 0.1	< 0.28*	< 0.25*	< 0.1	< 0.1	< 0.25
S15-100721	Three Seed Mix	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.37*
S15-100722	Savoury Seed Blend	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.81*
S15-100723	Sunflower and Pumpkin Seeds, Cranberries and Pine Nuts	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.27*
S16-001242	Milled Organic Flaxseed	< 0.1	< 0.1	< 0.1	< 1.92*	< 2.87*	0.11	< 0.1	< 0.25
S16-001243	Sunflower Seeds	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.4*
S16-001244	Black Chia	< 0.1	< 0.1	< 0.1	< 0.28*	< 0.38*	< 0.1	< 0.1	< 0.25
S16-003212	Super Seeds Flax Carob Bars	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.25

* Reporting limit raised due to interferences from high background signal or low recovery

(-) results in parenthesis were measured values but below the reporting limit

Table 26. Tropane alkaloid results for additional UK oilseed samples, in µg/kg (contd.)

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.25	0.1	0.1	0.1	0.5	0.1	0.1	0.1
Sample code	Product description	Conc (µg/kg)							
S16-003313	Milled Linseed	< 0.25	< 0.19*	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-003314	Golden Flaxseed Cold Milled Organic	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-003316	Sunflower Seed Kernels	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S15-100720	Brown Linseed	< 0.25	< 0.21*	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S15-100718	Golden Linseeds	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S15-100721	Three Seed Mix	< 0.25	< 0.1	< 0.1	< 0.1	< 0.65*	< 0.1	< 0.1	< 0.1
S15-100722	Savoury Seed Blend	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.16*
S15-100723	Sunflower and Pumpkin Seeds, Cranberries and Pine Nuts	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-001242	Milled Organic Flaxseed	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-001243	Sunflower Seeds	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-001244	Black Chia	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1
S16-003212	Super Seeds Flax Carob Bars	< 0.25	< 0.1	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1

* Reporting limit raised due to interferences from high background signal or low recovery

(-) results in parenthesis were measured values but below the reporting limit

Table 26. Tropane alkaloid results for additional UK oilseed samples, in µg/kg (contd.)

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	0.5	0.25	1.0*	0.5	0.5	0.06	0.5	0.5
Sample code	Product description	Conc (µg/kg)							
S16-003313	Milled Linseed	< 0.5	< 0.25	< 1.0	< 0.5	< 0.65*	< 0.06	< 0.5	< 0.5
S16-003314	Golden Flaxseed Cold Milled Organic	< 0.65*	< 0.25	< 1.18*	< 0.5	< 0.5	< 0.08*	< 0.5	< 0.5
S16-003316	Sunflower Seed Kernels	< 0.61*	< 0.25	< 1.0	< 0.5	< 0.67*	< 0.06	< 0.61*	< 0.68*
S15-100720	Brown Linseed	< 0.5	< 0.25	< 1.0	< 0.5	< 0.81*	< 0.06	< 0.5	< 0.5
S15-100718	Golden Linseeds	< 0.5	< 0.25	< 1.0	< 0.5	< 0.5	< 0.06	< 0.5	< 0.5
S15-100721	Three Seed Mix	< 1.22*	< 0.25	< 1.0	< 0.5	< 1.03*	< 0.06	< 0.5	< 0.5
S15-100722	Savoury Seed Blend	< 1.98*	< 0.25	< 1.0	< 0.5	< 1.1*	< 0.06	< 1.16*	< 0.58*
S15-100723	Sunflower and Pumpkin Seeds, Cranberries and Pine Nuts	< 0.5	< 0.25	< 1.0	< 0.5	< 0.76*	< 0.07*	< 0.5	< 0.5
S16-001242	Milled Organic Flaxseed	< 0.5	< 0.25	< 1.0	< 0.5	< 0.5	0.09	< 0.5	< 0.5
S16-001243	Sunflower Seeds	< 0.8*	< 0.25	< 1.0	< 0.5	< 0.99*	< 0.07*	< 0.5	< 0.5
S16-001244	Black Chia	< 0.5	< 0.25	< 1.0	< 0.55	< 0.5	< 0.08*	< 0.5	< 0.5
S16-003212	Super Seeds Flax Carob Bars	< 1.01*	< 0.25	< 1.0	< 0.5	< 0.68*	< 0.06*	< 0.79*	< 0.5

* Reporting limit raised due to interferences from high background signal or low recovery

(-) results in parenthesis were measured values but below the reporting limit

Table 27. Tropane alkaloid results for additional UK black and green samples, in µg/kg dry weight.

		Acetyl scopolamine	Anisodamine	Anisodine	Apo atropine	Apo scopolamine	Atropine	Convolamine	Convolidine
	Reporting limit (assigned LOD)	0.25	0.25	0.25	0.25	0.25	0.1	0.5	2.0*
Sample code	Product description	Conc (µg/kg)							
S16-003235	Pure Green Tea Unbleached Bags	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.1	< 0.5	< 2.0
S16-003236	English Breakfast Tea Bags	< 0.43*	< 0.25	< 0.25	< 1.22*	< 0.62*	< 0.19*	< 3.11*	< 2.0
S16-003237	Indian Fairtrade Tea	< 0.38*	< 0.25	< 0.25	< 1.14*	< 0.53*	< 0.1	< 2.74*	< 2.0
S16-003239	Pure Green Tea Bags	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.1	< 0.5	< 2.0
S16-003240	supreme green tea	< 0.25	< 0.25	< 0.25	< 0.34*	< 0.25	< 0.1	< 0.67*	< 2.0
S16-003241	Tea Temples Everyday Brew	< 0.46*	< 0.25	< 0.25	< 1.35*	< 0.61*	< 0.17*	< 3.55*	< 2.0
S16-003245	Organic Green Tea Bags	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	<0.1	< 0.5	< 2.0
S16-003248	Everyday Teabags	< 0.48*	< 0.25	< 0.25	< 1.69*	< 0.77*	< 0.17*	< 4.39*	< 2.0
S16-003249	Original Tea Bags	< 0.5*	< 0.25	< 0.25	< 1.59*	< 0.74*	< 0.1	< 3.87*	< 2.0

		Convolvine	Fillalbine	Homatropine	a-OH methyl atropine	6-OH tropinone	Littorine	Noratropine	Norscopolamine
	Reporting limit (assigned LOD)	0.5	0.5	0.3	0.5*	1.0	0.25	0.5*	0.25
Sample code	Product description	Conc (µg/kg)							
S16-003235	Pure Green Tea Unbleached Bags	< 0.5	< 0.5	< 0.3	< 0.5	< 1.0	< 0.25	< 0.5	< 0.25
S16-003236	English Breakfast Tea Bags	< 1.83*	< 1.92*	< 0.3	< 0.5	< 1.0	< 0.69*	< 0.5	< 0.25
S16-003237	Indian Fairtrade Tea	< 1.69*	< 1.64*	< 0.3	< 0.5	< 1.0	< 0.64*	< 0.5	< 0.25
S16-003239	Pure Green Tea Bags	< 0.5	< 0.5	< 0.3	< 0.5	< 1.0	< 0.25	< 0.5	< 0.25
S16-003240	supreme green tea	< 0.5	< 0.5	< 0.3	< 0.5	< 1.0	< 0.27*	< 0.5	< 0.25
S16-003241	Tea Temples Everyday Brew	< 1.99*	< 2.12*	< 0.3	< 0.5	< 1.0	< 0.8*	< 0.5	< 0.25
S16-003245	Organic Green Tea Bags	< 0.5	< 0.5	< 0.3	< 0.5	< 1.36*	< 0.25	< 0.5	< 0.25
S16-003248	Everyday Teabags	< 2.44*	< 2.23*	< 0.3	< 0.5	< 1.0	< 0.79*	< 0.5	< 0.28*
S16-003249	Original Tea Bags	< 2.41*	< 2.21*	< 0.3	< 0.5	< 1.0	< 0.82*	< 0.5	< 0.29*

* Reporting limit raised due to interferences from high background signal or low recovery

(-) results in parenthesis were measured values but below the reporting limit

Table 27. Tropane alkaloid results for additional UK black and green samples, in µg/kg dry weight (contd).

		Nortropinone	Phenylacetoxy tropane	Pseudotropine	Scopine	Scopoline	Scopolamine	Tropine	Tropinone
	Reporting limit (assigned LOD)	2.5	1.0*	2.5	1.0	1.0	0.1	2.5	1.0
Sample code	Product description	Conc (µg/kg)							
S16-003235	Pure Green Tea Unbleached Bags	< 2.5	< 1.0	< 2.5	< 1.0	< 1.0	< 0.1	< 2.5	< 1.0
S16-003236	English Breakfast Tea Bags	< 2.5	< 1.0	< 2.5	< 1.0	< 1.0	< 0.17*	< 2.5	< 1.0
S16-003237	Indian Fairtrade Tea	< 2.5	< 1.0	< 2.5	< 1.0	< 1.0	< 0.1	< 2.5	< 1.0
S16-003239	Pure Green Tea Bags	< 2.5	< 1.0	< 2.5	< 1.0	< 1.0	< 0.1	< 2.5	< 1.0
S16-003240	supreme green tea	< 2.5	< 1.0	< 2.5	< 1.0	< 1.0	< 0.1	< 2.5	< 1.0
S16-003241	Tea Temples Everyday Brew	< 2.5	< 1.11*	< 2.5	< 1.0	< 1.0	<0.1 (0.05)	< 2.5	< 1.0
S16-003245	Organic Green Tea Bags	< 2.5	< 1.0	< 2.5	< 1.0	< 1.0	< 0.1	< 2.5	< 1.0
S16-003248	Everyday Teabags	< 2.5	< 1.18*	< 2.5	< 1.0	< 1.0	< 0.15*	< 2.5	< 1.0
S16-003249	Original Tea Bags	< 2.5	< 1.15*	< 2.5	< 1.0	< 1.0	0.15	< 2.5	<1.0

* Reporting limit raised due to interferences from high background signal or low recovery

(-) results in parenthesis were measured values but below the reporting limit

FINAL REPORT

Occurrence of Tropane Alkaloids in Food - Literature Search

FS 112106

May 2016

Colin Crews and Susan MacDonald, Fera Science Ltd.

Annex 1. Literature review on Tropane Alkaloids in Food

Annex 1. Literature review on Tropane Alkaloids in Food

Tropane alkaloids Literature Search

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1. INTRODUCTION

The European Food Safety Authority (EFSA) has delivered a scientific opinion on the risks to human and animal health related to the presence of tropane alkaloids (TAs) in food and feed. Although more than 200 different TAs have been identified in various plants, data on their toxicity and occurrence in food are limited and the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) could only perform a risk assessment on (-)-hyoscyamine and (-)-scopolamine (EFSA 2013).

Results were reported on the levels of TAs in 124 food samples collected in the Netherlands and Germany. Most of the food samples (83 %) did not contain detectable TAs but almost all of the food samples that did contain TAs were in the food category 'Cereal-based food for infants and young children' especially those products intended to be to be reconstituted with milk or other appropriate nutritious liquids. They comprised simple cereals (wheat, maize, rye, oats, rice and mixtures thereof). The CONTAM Panel recommended that the occurrence of TAs in food either naturally or as contaminants be better characterised.

This report reviews the occurrence of TAs in food plants and in weed plants that both contain TAs and have a strong potential to contaminate foods. The objective was to provide a recommendation of the food samples to be analysed for TAs within the EFSA project.

The information was obtained by means of a literature search using the terms of an agreed strategy provided in the Appendix, and through studies in the Fera Information Centre and its available on-line sources. The search did not include literature that was not in the English language. TAs for which no standard were believed to be available, and cocaine and other TAs used for recreational drugs were excluded.

In view of the considerable quantity of data concerning the occurrence and toxicity of atropine and scopolamine and the EFSA interest in the remaining TAs the focus of the review was on the less familiar TAs including calystegines.

1.1 Chemistry of TAs

1.1.1 Tropanes

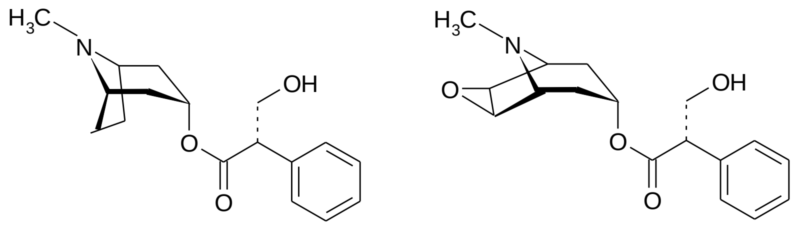
TAs are esters of hydroxytropanes (α-tropanol, α-tropane-diol or α-tropane-triol) with short-chain acids such as acetic acid, propanoic acid, isobutyric acid, isovaleric acid, 2-methylbutyric acid, tiglic acid, (+)-α-hydroxy-β-phenylpropionic acid, tropic acid and atropic acid. Atropine and scopolamine are esters of tropane-3α-ol (and the 6-7 epoxide of tropane-3α-ol) and tropic acid. Hyoscyamine is the ester of tropane-3α-ol (3α-hydroxytropane) and S-(-)-tropic acid.

The alkaloid part of TAs is a two-ringed structure characterized by a pyrrolidine and a piperidine ring sharing a single nitrogen atom and two carbon atoms. The asymmetric α-carbon of tropic acid esters allows the formation of two stereoisomers.

Hyoscyamine is synthesised in the plant as the optically active S-(-)- form and undergoes racemisation over time, so that both the R-(+)- and the S-(-)- forms are found in plants in varying ratio (Eich 2008). Analytical extraction procedures may affect the isomerisation and usually do not distinguish between the products and so in this review the name atropine will refer to the racemic mixture or an unspecified isomer.

TAs are synthesized in the young root cells and translocated to the aerial parts of the plant (Hashimoto *et al.*, 1991). The synthesis proceeds from ornithine and produces tropinone and then tropine (Zhang *et al.*, 2007) which is esterified to form littorine. Littorine is transformed to (-)-hyoscyamine by at least two routes and scopolamine is formed from (-)-hyoscyamine (Li *et al.*, 2006).

Tropinone reductases are key enzymes of TA formation. Two different tropinone reductases are involved, one forming tropine, leading to esterified alkaloids, and the other forming pseudotropine that is converted to the related TA known as calystegines (Dräger 1994, Keiner *et al.* 2000).

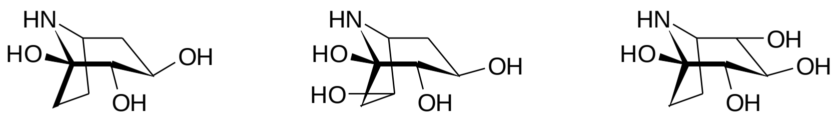


Atropine Scopolamine

Figure 1. Structures of atropine and scopolamine

1.1.2 Calystegines

Calystegines are polyhydroxy alkaloids with a nortropane skeleton. About 15 calystegines are known, and those most commonly reported include calystegine A3; A5; B1; B2; B3; B4; B5; *N*-methyl-calystegine B2; C1; C2; and *N*-methyl-calystegine C1. Not all of these are available commercially as reference standards.



Calystegine A3 Calystegine B1 Calystegine B2

Figure 2. Structures of the most common calystegines

2. IDENTIFICATION OF RELEVANT ASSOCIATED PLANTS:

To identify the TA containing plants that might contaminate food, cases of intoxication by TAs reported in the EU were studied and associated plants characterised. In addition the European Union's Rapid Alert System for Food and Feed (RASFF) was searched for alerts and notifications on TA or the presence of TA producing plants.

2.1 Reported cases of intoxication in the EU

Many reports of the acute effects of TAs in humans have resulted from deliberate ingestion in order to experience hallucinogenic effects (Boumba *et al.* 2004). Accidental ingestion of tropane-containing plants is also comparatively common. Seven people were poisoned by consumption of cooked wild collected spinach (blites) that contained leaves of *Datura innoxia* (Papoutsis *et al.* 2010). The cooked vegetables contained atropine and scopolamine at 0.8 and 1.2 mg/kg, respectively. The patients recovered with hospital treatment. Serious poisoning of a nine year old boy resulted from the ingestion of 20-25 *Atropa belladonna* berries (Lange *et al.* 1990), the symptoms being confused with a psychosis. A similar confusion surrounded the accidental poisoning of an elderly but healthy man with *A. belladonna* berries (Trabattoni *et al.* 1984). Three of four adults who ingested cooked ripened *A. belladonna* berries became delirious with visual hallucinations, one becoming comatose. One other adult and four children exhibited mild peripheral anticholinergic symptoms (Schneider *et al.* 1996).

Plant exposures are the fourth most common category of poisoning in Germany, accounting for 22% of paediatric exposures. 58,641 cases involving 248 different plant genera were reported from 1998 to 2004 of which about 10 % of cases had noticeable symptoms. In a separate German analysis of 111,313 calls to a regional poison control centre over a twenty year period from 1974, 56% referred to children and 44% to adults with plant ingestion being the cause of 9.7% of cases.

Analysis of severe poisonings by plants reported to the Swiss Toxicological Information Center from 1966 to 1994 showed that of 24,950 cases the clinical course was either mild or unknown (Jaspersen-Schib *et al.* 1996). Poisoning was severe in 152 cases which could be associated with 24 plants which included *A. belladonna* (42 cases), *Aconitum napellus* (4 cases) and *Hyoscyamus niger* (3 cases).

From 174 exposures of children toxic plants recorded in the Czech Republic over a 6-year period from 1996 to 2001 the most frequent cases (15%) resulted from ingestion of *Datura* seeds (Vichova *et al.* 2003).

Screening the literature for reports on food-related intoxications led to eleven cases concerning TAs described within the period from 1978 to 2010, as reported in a review by Adamse and van Egmond (2010). Five of these cases concerned documented or suspected contamination of different types of herbal teas; i.e. burdock (*Arctium*) root tea (Bryson *et al.*, 1978), nettle (*Urtica*) tea (Scholz and Zingerle 1980), two incidents with comfrey (*Symphytum*) tea (Galizia 1983, Routledge and Spriggs 1989) and Paraguay (*Ilex paraguariensis*) tea (CDC, 1995).

In the survey data reported in the EFSA Opinion of 2013 (EFSA 2013) of food samples from the Netherlands (112 samples) and Germany (12 samples) 93 were within the food group “Food for infants and small children” of which 19 samples in the “Simple cereals that are or have to be reconstituted with milk or other appropriate nutritious liquids” had mean atropine + scopolamine concentrations of 4.5 µg/kg at the Lower Bound limit and 4.9 µg/kg at the Upper Bound limit with the major contamination frequency (50%) being in the cereal products that were indicated specifically for toddlers (a total of 28) with food for both toddlers and infants, having lower levels in five products.

Datura is an invasive wild plant that appears to readily contaminate buckwheat (*Fagopyrum sagittatum*) and have serious health effects even at low doses (clinical manifestations were reported from ingestion of buckwheat seeds contaminated at a level of 190 seeds of *D. stramonium* per kg of buckwheat seeds (Perharič 2005). Buckwheat products are attractive to people who are intolerant of the gluten found in wheat.

In Slovenia in 2003, 73 cases of domestic food poisoning with mild to moderate effect were associated with ingestion of a traditional dish containing buckwheat flour, which is commonly used in Slovenia (Perharič 2005). Four samples of the flour were shown to contain atropine and scopolamine but it is not clear if the scope of analysis included other TAs. The whole buckwheat grain contained up to 190 seeds of *Datura stramonium* per kg of grain. Atropine and/or scopolamine was detected (> 3 µg/kg) in 20 of 43 buckwheat samples with the highest levels (26 mg/kg and 12 mg/kg of atropine and scopolamine respectively) found in the flour consumed by a family of eight people. The level of consumption was between 53 and 140 µg/kg body weight for atropine and between 25 and 64 µg/kg body weight for scopolamine. In Slovenia all buckwheat flour and buckwheat flour products were recalled and legislation on grain purity and buckwheat flour amended.

This incident was followed up by a survey of 75 samples of buckwheat grain and buckwheat food products collected in Slovenia (Perharič *et al.* 2013). The survey samples comprised 12 wholegrain buckwheat, 13 samples of groats, 34 flour, 8 pasta, 4 bread and 4 žganci (semi-prepared fermented buckwheat product) products. Fifty of the products were analysed by gas chromatography with mass spectrometry (GC-MS). 18 samples contained atropine and scopolamine at levels above the limit of quantification (LOQ) of 30 µg/kg. Of these eleven were flours, four were pasta, and three were “žganci”. The maximum contamination level of 26 mg/kg atropine and 12 mg/kg scopolamine was in buckwheat flour from Hungary. Of the 18 positive samples 11 were from Hungary, 4 from the Czech Republic, 2 from China and 1 from Slovenia. The TA content of the positive samples was 1.9 mg/kg atropine and 1 mg/kg scopolamine with a median content of 0.25 and 1.0 mg/kg, respectively.

An episode of buckwheat contamination was reported by Rancic and Spasic (2009). A large consignment (about 3000 kg) of buckwheat flour imported from a neighbouring country into Serbia in 2001 was analysed after a case of poisoning was reported. Seeds resembling those of *D. stramonium* were separated and analysed by automated multicolumn high pressure liquid chromatography (HPLC) with UV detection which confirmed the presence of atropine and scopolamine.

Atropine and scopolamine were found in buckwheat in France in 2007, and examination of the data in 2008 led to the setting a provisional threshold level of 0.02 mg/kg in flour intended for human consumption in France. The sum of atropine and scopolamine in the samples measured in 2007 was above 1000 µg/kg in ten samples with a maximum of 7.4 mg/kg. Five samples of buckwheat grain and 29 samples of buckwheat flour were measured in 2008 of which the sum of atropine and scopolamine exceeded 1 mg/kg in two samples (Afssa 2008).

A stock of Breton buckwheat that contained seeds of Datura caused poisoning in a large area of south-eastern France in September 2012 after it was purchased by a mill in the Alpes de Haute Provence (Glaizal *et al.* 2013). All of the victims had consumed bread, or in two cases homemade foods, prepared from organic buckwheat flour. The flour was sold in various bakeries and specialty food stores but was supplied by the same source, which was a grain company operating throughout the European Union. Tens of tons of this stock were distributed to four specialized organic mills in Brittany and to a lesser extent in Provence. Flour produced from the seeds was sold in over one hundred bakeries and was passed to retail outlets, organic shops, and restaurants. Contaminated flour or finished products such as black bread, and pancakes, etc. were distributed from the only mill located in Provence to not only the six departments of the Provence-Alpes-Côte d'Azur region but also to Corsica, Rhone -Alpes and Languedoc-Roussillon. Within less than 2 months, 24 people from the Provence-Alpes-Cotes d'Azur and Languedoc-Roussillon regions went to accident and emergency departments with symptoms of intoxication. Poisoning by buckwheat contaminated with Datura was confirmed in most (19) of these patients who were from 12 different households. In Rhone-Alpes, 14 people from 9 different households were intoxicated.

The distribution chain and chemical analysis linked the contamination of buckwheat to seeds of *Datura* sp. in the Breton area. Levels of atropine and scopolamine in the flour sent to the Provençal region were much higher (16,467 and 7,042 mg/kg respectively) than in other mills (no data were provided for Brittany). Cases of poisoning were recorded from some places over a month after the withdrawal and in Rhône-Alpes a case was reported after five months.

In 2011 Caligiani *et al.* (2011) measured atropine and scopolamine in buckwheat fruits, flours and retail foods sold in Italy. No TAs were found above an LOQ of 1 µg/g in buckwheat fruits (2), flours (7) or food products made from buckwheat (3 pasta, 2 porridge, 1 cracker, and 1 flakes).

Eight adults were poisoned in the Netherlands in 2013 in two or more incidents involving consumption of herbal tea prepared from dried marshmallow root (approximately 20 g), some of the cases were linked, and in all cases the tea came from the same supplier (van Riel *et al.* 2014). A similar case was reported in France with the herbal tea being purchased in The Netherlands. The atropine content of the tea was high (1–10 mg/g) and the plant source believed to be *Atropa belladonna* (deadly nightshade). The *Althaea officinalis was* harvested in Bulgaria and sold only in The Netherlands. The patients had anticholinergic symptoms (dry mucous membranes, nausea, blurred vision, hallucinations, tachycardia, and urinary retention) typical of tropane poisoning.

Frozen mixed vegetables contaminated with *Datura stramonium* were sold via the largest market chain in Finland in May of 2013 (Termala *et al.* 2014). A total of 28 poisonings were suspected and 10 patients had anticholinergic symptoms. The products were recalled and

30,000 customers were warned to avoid the vegetables. It was pointed out that the outbreak could have lasted much longer on account of the potential long storage time of the frozen product.

A single case of poisoning by a tea (described as a 'biodrug') in Germany, causing 'respiratory insufficiency' was reported by a physician in 2001 (BfR 2006).

2.2 Search of the EU RASFF system

A search of the RASFF Rapid Alert system produced relevant results from the search terms: “Atropine”, “Datura”, “Solanum” and “Hyocyamus”. No results were obtained for the names of other TAs or other TA-producing plants. All results for “Scopolamine” were also returned under the “Atropine” search.

The RASFF system contained notifications and/or alerts for atropine, scopolamine, *Datura*, *Solanum*, and *Hyoscyamus* as shown in Table 1.

Datura and atropine/scopolamine

Six reports to RASFF concerned the occurrence of atropine and scopolamine in buckwheat (*Fagopyron esculentum*) flour. All were reported by Slovenia. Two in 2006 reported atropine (35-37 µg/kg) and scopolamine (48-65 µg/kg) in flour from Ukraine and the Czech Republic. One in 2009 reported atropine (110 µg/kg) and scopolamine (47 µg/kg) in raw material from Hungary, one in 2012 reported atropine (20 µg/kg) and scopolamine (6 µg/kg) in flour from Slovenia, and two in 2013 reported atropine (14-18 µg/kg) and scopolamine (6-11 µg/kg) in buckwheat flour from Austria prepared from raw material from Slovakia and the Czech Republic.

In addition there were seven reports of atropine contamination in millet, usually with scopolamine.

Between 2006 and 2014 there were seven RASFF Notifications or Alerts of *D. stramonium* seeds in food of which six related to millet. A level of 130 µg/kg was reported in 2006 in organic millet from Austria and from Hungary. A second case concerned seeds found in brown millet (*Uroclora ramosa*) from Austria, three further notifications were for *Datura* seeds in organic millet from Austria. *Datura* seeds were also found in vegetable and bacon stir-fry mix from Spain, in 2013 in frozen vegetable-bean-seed mix from Belgium made from raw material produced in Spain, and in 2014 in organic baby food (apple, pear and millet) manufactured in Germany. The baby food contained atropine at levels between about 4 and 32 µg/kg. In one of the 2006 incidents in Austria, a dish made from millet and carrot that was contaminated with seeds from *D. stramonium* was responsible for poisoning eight people, of whom one required hospitalisation. The millet contained about 50 seeds/kg grain (Fretz *et al*. 2007).

D. stramonium fruits (presumably unripe seed cases) were found in canned green beans from Hungary in 2006 and again in 2007 and also in beans from Spain in Finland in 2013.

Solanum

In 2015 plant material of *Solanum nigrum* was rejected at the border of Portugal after the attempted import of food supplements from India. *Solanum nigrum* fruits were reported in 1982 in frozen green beans (from France) sold in Greece, in 1985 in beans in Germany, in 2004 in two samples of frozen green beans in France, and in 2013 in young green beans from the Netherlands.

Hyoscyamus

Seeds of *Hyoscyamus niger* (henbane) were found as contaminants in poppy seed from the Czech Republic in 2007 and 2008. The contamination level was between 0.13 % and 0.42 %. Two of the samples were sold in the Slovak Republic and the third in the Czech Republic.

2.3 Literature search results for plant species that could potentially contaminate field crops in the EU.

Plant species that could potentially contaminate field crops in the EU were identified from the search results and from searches of databases of weed science organisations. Plants identified in these searches that were designated problematic as agricultural weeds and are also known to contain TAs are described below.

In addition to the searches carried out using the Fera Information Centre the databases of and reports issued by the following weed science organisations were searched:

The European Network on Invasive Alien Species (NOBANIS) (http://www.nobanis.org/)

The Global Biodiversity Information Facility (http://www.gbif.org/)

The International Weed Science Society (http://www.iwss.info/)

The European Weed Research Society (EWRS) (http://www.ewrs.org/)

The EWRS Invasive Plants Working Group.

The EWRS Weed Mapping Working Group.

Association Française de Protection des Plantes (conference proceedings)

Sociedad Española de Malherbología (http://www.semh.net/)

The Italian Weed Research Society

The Plant List maintained by the Royal Botanic Gardens, Kew and Missouri Botanical Garden (http://www.theplantlist.org/)

2.4 Results

The major plant species that contaminate field crops in the EU or had a strong potential to do so were identified as *Solanum* and *Convolvulus* species with a lesser risk from *Brassica, Atropa* and *Datura* species.

Solanum species

Solanum nigrum, known as the 'Common nightshade or "Black nightshade' was the most frequently reported *Solanum* weed although confusion with related similar species is very likely.

It is an annual weed growing to 0.6 x 0.3 m. which flowers in late summer. It produces fruits that are at first green and might be unseen in vegetables. These turn black on maturity and might then be mistaken as edible fruits. *S. nigrum* ranked joint 13th in a comparison of the overall importance of European weeds published by Schroeder *et al.* (1993). It ranked 6th in importance in relation to infestation of maize and sorghum.

Solanum elaeagnifolium known as ‘yellow tomato’ or ‘silverleaf nightshade’ is a highly infective toxic weed of vegetable crops, cotton and pastures in the eastern Mediterranean countries (Boyd *et al.* 1984, Mekki 2007). It is considered one of the worst weeds in the world. *S. elaeagnifolium* became invasive in many countries after it was unintentionally introduced to Europe and is now widespread in or considered a risk to Albania, Algeria, Bosnia and Herzegovina Bulgaria, Croatia, Cyprus, France, Georgia, Greece, Hungary, Israel, Italy, Jordan, Kazakhstan, Moldova, Malta, Morocco Portugal, Spain, Romania, Russia, Serbia and Montenegro, Slovenia, Tunisia, Turkey, Ukraine and Uzbekistan. It colonizes roadsides, pastures and grasslands and infests crops. Maize, sorghum and wheat are considered to be very affected by *S. elaeagnifolium* which can be abundant in fields. In Morocco it has caused serious damage to maize crops (Gmira *et al.* 1998, Taleb and Bouhache 2006). According to a report from the European and Mediterranean Plant Protection Organisation (EMPPO 2006) the plant is present in the five continents and in several countries where it is invasive.

Solanum plants that have weed status in Russia with the potential to spread westwards are: *Solanum carolinense* L. (horse-nettle) and *Solanum cornutum* (buffalobur). *S. carolinense* is a native of the USA but spreading in Russia. It is an extremely competitive weed that infests corn and other grain crops, potato, soybean, and tomato. (AgroAtlas Project 2009). *S. cornutum* is a native of Mexico and the southwest USA now found in the European part of the Former Soviet Union, and described as a pernicious quarantine weed of spring grain crops, poisonous to animals (AgroAtlas Project 2009).

Convolvulus species

Convolvulus arvensis (Field bindweed) is considered one of the worst weeds in the world by agriculturists and horticulturists (Austin 2000). It grows very successfully in temperate and Mediterranean climates where it is a weed of cereals, beans and potatoes. It is problematic in France, Germany, Greece, the former Yugoslavia, and many countries outside Europe. *C. arvensis* ranked joint 7th in a comparison of the overall importance of European weeds, 15th in the rankings for its importance in spring cereals crops and in winter rapeseed, and 6th in maize and sorghum (Schroeder *et al.* 1993, Schimming *et al.* 2005, Shukla *et al.* 2006).

Kaçan *et al.* (2013) described weeds as a major obstacle to sunflower seed production in Turkey. *C. arvensis* was among the major weeds found in 2012.

Brassicaceae

Cochlearia species (scurvy grass) are annual Brassicaceae plants that do not have an impact on crops but were the first brasssicas to be shown to contain tropanes. Calystegines were found in two species (*Berteroa incana* and *Bunias orientalis*) that can be invasive weeds. *Berteroa incana* is an invasive and toxic weed, especially of alfalfa and clover forages, although the identity of the toxin has not been confirmed. *Bunias orientalis* is a brassica native to the Caucasus and southern Russia. It has spread rapidly throughout Europe through the human use as animal feed is now naturalized in many countries in Europe and is still spreading intensively (Birnbaum 2006), especially in Estonia where it is listed as particularly invasive and fast-spreading.

Calystegines were also found in two minor weeds (*Barbarea vulgaris* and *Bunias erucago*) that have been proposed as human salad or vegetable foods (PFAF 2015), and in a number of other brassicas (*Arabidopsis thaliana, Coringia orientalis, Cronopus squamatus, Diplotaxis murales, Isatis tinctoria, Lunaria annua, Lunaria rediviva, Matthiola incana, Neslia paniculata, Peltaria alliacea, Sisymbrium strictissimum*) that can probably not be considered a health threat.

Brassica tournefortii (Sahara mustard, African mustard, Asian mustard, Mediterranean turnip, Sahara mustard, Tournefort's birdrape, wild turnip, is a native annual herb in Europe, common in disturbed sites such as roadsides and abandoned fields, but is not considered invasive in Europe. *B. tournefortii* is found in the Middle East and in the European and neighbouring countries Azerbaijan, Cyprus, Egypt, Greece, Italy, Morocco, Spain, Tunisia, Turkey, Turkmenistan and Uzbekistan. It is widely used as food in some North African countries.

Brassica elongata (elongated mustard, long-stalked rape) is a biennial or perennial herb that is native to Eastern Europe, Russia and Central Asia. In some areas it competes with native flora and has the potential to spread. *B. elongata* is cultivated in Estonia (USDA ARS 2006).

Brassica campestris L. (Wild turnip)

This plant is generally distributed as a weed in temperate zones of Europe and is cultivated plant in parts of the Middle East and Asia. It is found throughout the Western part of Russia, in the Caucasus, Western and in Eastern Siberia. It has been described as a pernicious weed infesting all spring crops, both grain and vegetative types (AgroAtlas Project 2009)

Datura

Datura is a frequent contaminant of food crops as is evidenced by the reports under the RASFF system. However there were no further descriptions of the scale of the associated weed problem in the results from the search. *Datura stramonium* was among the major weeds described as a major obstacle to sunflower seed production in Turkey in 2012 (Kaçan *et al.* 2013).

Hyoscyamus albus

This is the henbane plant which has been described as a natural weed in much of Europe (Williams 1982, Hanf 1983, Williams and Hunyadi 1987).

Others

Atropa belladonna and *Physalis* species contain TAs but are not reported as troublesome weeds in Europe. *Mandragora autumnalis*, the main Mediterranean species of the mandrake plant contains many toxic tropanes (Hanuš *et al.* 2005) but is not particularly effective as a weed. *Hyoscyamus niger* (Black Henbane) is a weed of poppy, wheat, and millet crops. This plant produces very many seeds, up to 400,000 per plant (AgroAtlas Project 2009). The literature search did not provide information on the scale of the associated weed problem but again the RASFF reports indicate a significant degree of food contamination.

The Australian native trees *Duboisia leichhardtii* and *D. myrporoıdes* that grow well in the warmer parts of Europe contain high concentrations of atropine-like alkaloids especially atropine and scopolamine but the trees are too few in number to cause a weed problem.

3 IDENTIFICATION OF RELEVANT FOOD PLANTS WITH INHERENT TA:

Adamse and van Egmond (2010) listed a number of TAs in food with examples of international poisoning incidents based on misidentification. Most food plants that naturally contain TAs belong to the Solanaceae family, which is one of the most diverse and varied groups of plants with 90 genera and just over 4000 species. It is found worldwide with the majority of species in Central and South America. Food plants of the Solanaceae family relevant for food consumption in the EU were identified. They are listed in Table 2.

3.1 Food plants of the Solanum family with relevance for food consumption in the EU

Plants of the genus Solanum do not contain atropine or scopolamine (Griffin and Lin 2000).

Potato *Solanum tuberosum*

Potatoes similarly originated in South America and have become a staple item in the European diet since the 18th century.

Tomato *Lycopersicon esculentum*

Tomatoes were imported from South America to Europe in the 15th century and has since been grown and hybridised extensively in Europe. The cultivated *L. esculentum* can be hybridised with *S. lycopersicoides*, *S. ochrantum* and *S. sitiens*.

Aubergine or eggplant *Solanum melongena*

Aubergines are believed to originate in Asia or Africa but it has been grown in the Mediterranean basin since the 7th century. Several minor species physically similar to aubergine are cultivated on a small scale for food, including scarlet and gboma eggplants (*S. aethiopicum* and *S. macrocarpon*)

Pepper *Capsicum* spp

Peppers comprise very many species of which five - the sweet bell pepper (*C. annuum*) and four ‘chilli’ types: *C. frutescens, C. chinense, C. baccatum* and *C. pubescens* have been domesticated.

Lesser solanums:

Cape Gooseberry *Physalis peruviana*

The Cape Gooseberry (*P. peruviana*) is a popular fruit in much of Europe but not eaten in quantity. This plant and the related *P. alkekengi* (Chinese lantern, bladder berry) contain several tropane and secotropane alkaloids in the roots and/or leaves (Griffin and Lin, 2000) but there are no reports of the TAs in the edible parts (fruits). The roots of *P. alkekengi* contain the minor TAs tigloidine, 3α-tigloyloxytropane, cuscohygrine and phygrine over the range 0.02 to 0.025 % fresh weight or 0.084-0.104 % dry weight (Basey and Woolley, 1973).

Tamarillo or Tree Tomato *Solanum betaceum*

This is popular in New Zealand where it is eaten raw as a fruit or vegetable dish and also cooked, but is receiving attention in Europe. Some related species also have edible fruits. It has not been reported to contain TAs.

Tomatillo *Physalis philadelphica or Physalis ixocarpa*

This fruit is becoming popular in Europe as a result of interest in Mexican foods. Its cultured root has been shown to contain calystegine A3 but not A5, B1 or B2 which were present in related species (Azemi *et al.* 2006).

Goji berry (*Lycium barbarum* and *L. chinense*)

The fruits of *Lycium barbarum* (goji berries) plants have recently been marketed as foods beneficial to health. Plant parts of *Lycium* have a history of use for medicinal purposes in China but in the West usage is restricted to the berries of *L. barbarum* (Pottera 2010). The berries are generally produced in and exported from China but the plant is believed to originate from the Mediterranean Basin (Genaust 1996) and might be cultivated in Europe in future.

Others

Solanum muricatum (pepino), *S. betaceum* (tree tomato), and *S. quitoense* (naranjilla) are popular in Mexico and South America and have potential to become popular in Europe, as does *S. melanocerasum* (garden huckleberry).

3.2 Food plants of the *Convolvulaceae* family with relevance for food consumption in the EU

The most relevant food plants of the Convolvulaceae are *Ipomoea*, of which about 700 species are found in most tropical and subtropical regions. The most important plant is the sweet potato or yam (*I. batatas*). Sweet potatoes are grown in many countries and consumed throughout most of the world (Zhao *et al.* 2005, Bovell-Benjamin, 2007).

Another ipomoea, *I. aquática* is a food crop in much of Asia, where the leaves are consumed as a rich source of nutrients and essential amino acids. Some related ipomoea species eaten to a lesser degree are *I. alba L., I. albivenia, I. involucrata* and *I. leptophylla*.

3.3 Food plants of the Brassica family with relevance for food consumption in the EU

Many Brassicaceae plants are part of the staple European diet, particularly those from the species *B. oleracea*, including broccoli, cauliflower, kale and Brussel sprouts. Other commonly consumed brasssicas are *B. napa* (turnip, white turnip) *B. rapa* (swede, rutabaga, Chinese cabbage), the colloquial names of which vary across Europe. Other Brassicas are consumed to a lesser but growing extent in Europe, including kohlrabi (*B. oleracea*, *Raphanus sativus*), rocket (*Eruca sativa*) and watercress (*Nasturtium officinale*). In addition many are consumed at lower level as ingredients or spices, such as brown mustard (*B. juncea*), black mustard (*B. nigra*), white mustard (*Sinapis alba*), or as sauce components such as horseradish (*Armoracia rusticana*) and wasabi (*Wasabi japonica*).

3.4 Other plants

The leaves and fruits of the white mulberry tree *Morus alba* have been shown to contain calystegines (Asano *et al.* 1994, Singhal *et al.* 2010, Bajpai *et al.* 2012, Singh *et al.* 2013). The white mulberry is a tree of economic importance in Asia as the leaves are the sole food source for silkworms and they are sometimes eaten as vegetable. *M. alba* is considered a weed in the USA and is found in warmer European countries. The fruit is consumed in Asia and the USA and is becoming popular in Europe, although the red (*M. rubra*) and black (*M. nigra*) species are preferred. Calystegines have not been reported in these two species.

Summary

In summary the most important food plants that naturally contain TAs have been identified as the Solanums (potato, aubergine and pepper), and the calystegine containing brasssicas (cabbage, broccoli) and sweet potatoes.

4 ASSESSMENT OF THE TA

4.1 TA in the food plants

TA in the food plants

Overviews of the TA in the food plants and associated plants were provided by Draeger *et al.* (1995), Griffin and Lin (2000) and by Pomilio *et al.* (2008). Draeger *et al.* (1994) and Asano (2000) surveyed the occurrence of calystegines in edible fruits and vegetables, and detected some or all of calystegines A3, B1, B2, and C1 in samples of the Solanaceae and Convolvulaceae tested (Asano *et al.*, 1997a, 1997b). A review of the occurrence of calystegine alkaloids in food plants was prepared by Watson *et al.* (2000) who reported their presence in over 70 varieties of potatoes other edible species such as aubergine and sweet potato.

The presence of calystegines in human foods such as potatoes, eggplants, and sweet potatoes poses the question as to the effect that these compounds might have on humans.

Calystegines in food plants

4.1.1 Potatoes

Calystegines have been found in all edible solanaceous plants (Asano *et al.* 1997a), and potato plants contain calystegines in all parts. They have been measured in over 70 edible varieties of potatoes (Nash *et al.* 1998, Watson *et al.* 2000, Friedman *et al.* 2003). Potatoes measured by Dräger (2004) contained calystegines at up to 2,300 mg/kg fresh weight in their sprouts and up to 450 mg/kg in the peel of the tuber.

Nash *et al.* (1993) reported a total calystegine level of 10 mg/kg fresh weight in the tuber flesh and Asano *et al.* (1997a) reported 3.4 to 7 mg/kg total tuber fresh weight. Calystegine A3 and B2 were measured in potatoes at a total alkaloid content of 3.5 to 7 mg/kg fresh weight (Watson *et al.* 2000). The ratio of B2:A3 was typically 2:1.

Keiner and Dräger (2000) reported details of the variation of calystegine levels in various parts of a single cultivar (Liu) of *S. tuberosum* during the life cycle of the plant. In the mature plant total calystegines reached 25 to 75 mg/kg in the leaves and about 100 to 250 mg/kg in the growing tuber. Directly after harvest the tubers contained a maximum of about 400 mg/kg calystegines in the eyes and skin, which rose to maxima at 5 months after harvest to 1000 mg/kg in the skin and 3000 mg/kg in the young sprouts emerging from the tuber when stored in the dark at +4˚C. Calystegines A3, B2 and B4 were the most prominent and could be quantified in all tissues (roots, tubers, leaves, sprouts and eyes), and calystegines A5, B1 and B3 were believed to be present in very small quantities (< 10 mg/g fresh weight). N-methyl calystegine B2 was not detected in any part.

Contrary to potato glycoalkaloids, calystegine levels in the skin do not increase on exposure to light but actually decrease. The major changes in calystegine levels during tuber production and post-harvest probably account for the variations in the levels reported in the literature.

Calystegines in potato (and probably other vegetables) are stable through cooking and other food processing operations (Watson *et al.* 2000). Storage of potatoes at 5 °C increased the proportions of the 4-*O*-alpha-D-galactoside of calystegine B2 and the trihydroxylated calystegine A3.

Watson et al (2000) reported the calystegine content of seven prepared potato products cooked following the recommended domestic procedures. All products contained calystegines A3 and B2. The highest levels were in French fries for microwave cooking (A3 and B2 at 15 and 20 mg/kg fresh weight respectively), instant mashed potato (9 and 23 mg/kg respectively), and potato crisps (5 and 21 mg/kg respectively).

4.1.2 Aubergines

TAs have been reported in the fruit of the aubergine or eggplant (Nash *et al.* 1993, Das and Barua 2013). The fruit contained calystegine A3 at 0.3 and B2 at 0.5 mg/kg fresh weight (Asano *et al.* 1997a). There has been some interest in the grafting of food plants on to the root stock of related species for food production. There is a single report of poisoning associated with consumption of aubergine that that been grafted on to the root of *Datura metel* (Oshiro *et al.* 2008).

4.1.3 Peppers

Capsicum annuum has been found to contain high levels of calystegines. Asano *et al.* (1997a) reported calystegines B1, B2 and C1 at 12, 37, and 4 mg/kg fresh weight respectively in C. annuum, but no A3. Chili pepper (*C. frutescens*) contained 0.24 mg/kg A3 and 0.27 mg/kg B2 but no A3 or C1.

4.1.4 Brasssicas

Brassicaceae species used as food do not contain atropine or scopolamine (Griffin and Lin, 2000), however the leaves of the majority of Brassicaceae contain calystegines. Calystegines are accumulated throughout the life cycle of brassica plants and are found in all parts.

Brassica oleracea (broccoli) has been reported to contain calystegines at concentrations up to 30 mg/kg dry weight (Dräger, 2004, Brock *et al.*, 2006). Calystegine A3 was present at 15 mg/kg and A5 at 4 mg/kg; *B. rapa* (turnip/oriental cabbage) contained A3 at 20 mg/kg and A5 at 3 mg/kg; *B. oleracea* (Brussel sprout) contained A3 alone at 5 mg/kg; and *B. nigra* (black mustard) contained A3 at 1 mg/kg, A5 at 5 mg/kg, B2 at 3 mg/kg and B3 at 4 mg/kg (Schimming *et al.* 2005).

4.1.5 Sweet potatoes

The Convolvulacea contain convolvine (3α-veratroyloxynortropane), and convolidine (3α-vanilloyloxynortropane) (Griffin and Lin, 2000). In a survey by gas chromatography/mass spectrometry (GC-MS) of the composition of 129 convolvulaceous species belonging to 29 genera (all 12 tribes) Schimming *et al.* (2005) found from one to six calystegines in 62 species belonging to 22 genera of all tribes except *Cuscuteae*. *Ipomoea* had the highest number of calystegine-positive species. Calystegines B1, B2, B3 and C1 have all been reported in the sweet potato *I. batatas*, as well as in the related *I. alba, I. aquatica,, I. carnea, I. eremnobrocha, I. obscura, I. pes-caprae, I. setifera, I. violacea* and *I. hederifolia* (B1, B2, C1 only).

The levels of calystegines reported in the edible *I. batatas* tuber by Asano *et al.* (1997a) were B1 (2.4-16 mg/kg) and B2 (1.1-19 mg/kg fresh weight).

4.1.6 Cape Gooseberry

The Cape Gooseberry *Physalis peruviana* contains no scopolamine, hyoscyamine, littorine or ditigloyl esters. It does contain tigloidine (3-β-tigloyloxytropane) and 3-α-tigloyloxytropane in the root (Beresford and Woolley 1974, Griffin and Lin 2000). The roots also contain the secotropane alkaloids (+)-physoperuvine, racemic physoperuvine and (+)-N,N-dimethyl-physoperuvinium, which are metabolites of TAs (Sahai and Ray, 1980). Both leaves and roots contain tropine, 3-β-tigloyloxytropane, 3-β-acetoxytropane, hygrine, two isomers of N-methylpyrrolidinylhygrine, physoperuvine, and cuscohygrine (Kubwabo *et al.* 1993). The related *P. alkekengi* contains calystegine B2. In analyses by Asano *et al.* (1997a) *Physalis peruviana* fruit contained calystegines A3 at 0.003 mg/kg, B1 at 0.038 mg/kg and B2 at 0.048 mg/kg fresh weight.

A study of the potential of the root of *P. peruviana* root for use as a functional food found that it contained cuscohygrine, withanolides and flavonoids. Five compounds were isolated and their structures elucidated by different spectral analysis techniques. Animal experiments showed that root extracts improved oxidative stress, liver function and kidney disorders, protecting the liver and kidney against fibrosis (El-Gengaihi *et al.* 2013).

4.1.7 Goji berry

The fruits from an Indian goji berry plant have been reported to contain atropine at a level 9.5 g/kg and hyoscyamine at 2.9 mg/kg dry fruit at (Harsh 1989) but this degree of contamination would have led to poisoning incidents. A later investigation of the atropine content of eight samples of goji berries from China and Thailand based on liquid chromatography-mass spectrometry (LC-MS) found atropine in one sample of dried berries at 0.019 mg/kg whereas for seven others the level was <0.01 mg/kg (Adams et al. 2006). A further evaluation by the German Federal Institute for Risk Assessment (BfR) published by Klenow *et al.* (2012) concluded that the original test was not specific for tropanes and the plant was probably misidentified *Lycium europaeum*. Analysis of the two species by liquid chromatography-tandem mass spectrometry (LC-MS/MS) showed an atropine content of 0.59 mg/k g for fruits of *L. europaeum* and atropine at the detection limit (0.01 mg/kg) in the fruits of *L. barbarum* from China (Klenow *et al.* 2012).

The roots of *L. chinense* contain at least 14 calystegines and N-methylcalystegines and other compounds with β-glucosidase and/or α-galactosidase inhibitory activity (Asano *et al.* 1997b).

4.1.9 White mulberry

The leaves and fruits of the white mulberry tree *Morus alba* have been shown to contain calystegines but they have not been found in the red or black varieties *M. rubra* and black *M. nigra* (Singhal *et al.* 2010, Bajpai *et al.* 2012, Singh *et al.* 2013). Asano *et al.* (2001a) found 17 polyhydroxylated alkaloids in the plant including calystegine B2 in the leaves, 2-hydroxymethyl-3,4-dihydroxy-pyrrolidine-*N*-propionamide in the root bark and 4-O-α-D-galactopyranosyl-calystegine B2 and 3β,6β-dihydroxynortropane in the fruit.

4.1.10 Other plants

The berries of *Atropa belladonna* have been mistaken for edible berries such as bilberries, blueberries, cranberries, and huckleberries, and the leaf has been mistaken for *Malva sylvestris* (mallow), used occasionally as a vegetable (Adamse and von Egmond 2010). Several parts (leaves, seeds and roots) of other plants such as Datura have been mistaken for common vegetables and herbs.

4.2 TA in the associated plants

Calystegines in associated plants

4.2.1 Datura species

The TA profiles of Datura species have been much studied and over 65 TAs have been reported. Most of the information relates to atropine and scopolamine which predominate in all species investigated (Berkov *et al.*, 2006, Doncheva *et al.*, 2006, Philipov *et al.*, 2007). TA patterns in Datura are influenced more strongly by environmental factors than by genetic ones, but the proportion of the less common TAs in the seed of European Datura is low compared to those of atropine and scopolamine. Ionkova *et al.* (1994) reported quite high levels of apoatropine (3α-apotropoyloxytropane) in the seeds of a cultured plant (calculated as 27 % of the gas chromatography-mass spectrometry response). Very few other TAs were reported in seeds in these studies, with the greatest variety of TAs being in the leaf tissue, It is likely therefore that atropine and scopolamine are the metabolic end products for storage in the seed.

4.2.2 *Atropa belladonna*

Atropa belladonna contains mainly atropine and scopolamine both in leaves and seeds, with lesser concentrations of apoatropine (Dhar and Bhat, 1982, Martinsen *et al.* 1991, Ashtiania and Sefidkonb 2011) and traces of tropine, belladonnine and, 6-β-hydroxyhyoscyamine (Arraez-Roman *et al.* 2008), tigloytropeine, aposcopolamine, hydroxyhyoscyamine and tigloyloxytropane (Gaillard and Pepin 1999), and possibly littorine (Nakanishi *et al.* 1998). Also reported present are scopolamine and apoatropine (Evans 1979), hyoscyamine N-oxide and scopolamine N-oxide (Phillipson and Handa 1975).

Tropanes are present in all parts of the plant but the total TA levels vary considerably between different varieties and harvesting stages (Dhar and Bhat, 1982). Atropine increases during growth with apoatropine appearing later (Clair *et al.* 1976). Atropine and scopolamine are always the major TAs (Simola *et al.* 1988) with atropine always the most abundant, up to 10 mg/g dry weight in the root and 3 mg/g in the leaves. Scopolamine was present at 0.6 mg/g in the root and 0.4 mg/g in the leaves of plants measured by Zarate *et al.* (1997). Fresh plants from Iran *A. belladonna* L. contained about 3% total TAs in the leaves 8 % in the roots, 1 % in the stem and 5 % in the seeds (Ashtiani and Sefidkon 2011). Hyoscyamine *N*-oxide is produced with the highest concentrations in the fruit (Phillipson and Handa 1973, 1976). An overview of quantitative data available in the literature is given in the EFSA Opinion (EFSA 2013). The range of total atropine + scopolamine was 20 to 5,000 mg/kg in leaves and 960 to 7,300 in seeds.

A. belladonna also contains calystegines (Dräger 1993, 1994, 1995). Calystegines were located in all parts but mainly in the young upper leaves (Dräger *et al.* 1995) where the levels vary but can reach about 400 mg/kg dry weight (Bekkouche *et al.* 2001). The presence of calystegine B4 is unconfirmed.

4.2.3 *Hyoscyamus niger*

Atropine and scopolamine are also the major TAs in *Hyoscyamus niger* which unusually has a higher scopolamine level than that of atropine. *H. muticus* (Egyptian henbane) is particularly rich in TAs (Oksman-Caldentey *et al.* 1987, Mandal *et al.* 1991). *Hyoscyamus albus* contains calystegines A3, B2, B3 and B1 in its cultured roots (Dräger *et al.* 1994).

4.2.4 *Convolvulus arvensis*

The TAs of *Convolvulus arvensis* have been studied by Asano *et al.* (1997a) and Asano (2000) and others. Early studies (Evans and Somanabandhu 1974, Jennet-Siems and Eich 1994, Todd *et al.* 1995 reported tropine, pseudotropine, tropinone, ferulyl tropinol and 3β-tropinol. De Simone *et al.* (2008) found that the roots of *C. arvensis* contain calystegines A3, A5, B1, B2, B3 and B4. The most frequently encountered calystegines were A3, B1, B2 and B3, while distribution of N1 and C1 was more limited. Bekkouche *et al.* (2001) did not detect A5 or B4. De Simone *et al.* (2008) found the related compounds convolidine (3α-vanilloyloxynortropane), convolamine (*N*-

methyltropan-3α-yl-veratrate), confoline (3α-veratroyl-*N*-formylnortropine), confolidine ((±)3a-vanillyl-*N*-formylnortropane, and convolamine-*N*-oxide.

Fresh aerial parts of *C. arvensis* plants which had poisoned horses were not shown to contain calystegines (Todd *et al.* 1995) but pseudotropine (a biosynthetic precursor of the calystegines) meso-cuscohygrine, a cuscohygrine stereoisomer and traces of atropine and tropinone were found. *C. arvensis* contains hygrine (Evans and Somanabandhu 1974, Jennet-Siems and Eich 1994, Todd *et al.* 1995), ferulyl tropinol, tropine, pseudotropine and 3-β-tropinol (Jennet-Siems and Eich 1994, Todd *et al.* 1995). Todd *et al.* (1995) found no calystegines in the aerial parts but Schimming *et al.* (2005) reported calystegines A3, A5, B1, B2, and B4, with the flowers containing only A5 and B2. Bekkouche *et al.* (2001) reported that the most frequently encountered calystegines were A3, B1, B2 and B3, while distribution of N1 and C1 was more limited. Calystegines A5 and B4 were not detected.

4.2.5 Solanum dulcamara

Solanum dulcamara was reported to contain calystegines A3, B1 and B2 by Asano *et al.* (2001b).

Camelina sativa, (camelina), a weed of flax seed, also contains calystegines A3, A5, B2 and B3, at levels of 1 to 5 mg/kg (Schimming *et al.* 2005).

4.2.6 Plants that contain TAs but are not considered to be likely to contaminate food

Mandragora - A number of poisonings by the mandrake *Mandragora autumnalis* have been reported in Italy, the native habitat for this plant (Piccillo *et al.* 2002). However it is not a seriously invasive weed. *M. autumnalis* and *M. vernalis* contained hyoscyamine, hyoscine, cuscohygrine, apoatropine 3 alpha-tigloyloxytropane and 3,6-ditigloyloxytropane. Belladonnine was present in the dried roots, but could not be detected in fresh roots (Jackson and Berry 1973).

Scopolia - Contains several TAs but is not seriously invasive (Asano *et al.*, 1996).

Duboisia - *Duboisia leichhardtii* is a tree cultured in Australia the leaves of which contain calystegines C1 and C2 (Kato, et al. 1997), but the plant is not considered invasive in Europe.

4.3 Available toxicity data of individual TAs

Toxicity data for the TA compounds can be considered more reliable than that obtained for plant poisoning as several of the plants described in this review contain toxins in addition to the TAs, in particular potatoes contain glycoalkaloids often cited as the active agent.

4.3.1 Atropine type TAs

The atropine type TAs are anticholinergic compounds and the poisoning symptoms are due entirely to inhibition of muscarinic acetylcholine receptors in the central nervous system (CNS) and the autonomic nervous system (ANS). Ingestion of TAs causes a variety of symptoms including: a dry mouth and upper digestive and respiratory tracts, hot red skin, pupil dilation (mydriasis) and blurred vision, changes in heart rate, tachycardia, and respiratory depression, urinary retention, ataxia, speech disturbance, disorientation and visual hallucinations. Studies in mice have shown no clear evidence of teratogenicity (Arcuri and Gautieri 1973). Atropine and scopolamine are not mutagenic in bacterial assays (EMEA, 1998).

The metabolic pathways of atropine and scopolamine differ. Atropine has a high oral bioavailability (Lewis and Elvin-Lewis, 1977) whereas scopolamine does not (Ali-Melkkilä 1993). Data for other TAs are not available. Following absorption, both alkaloids have a high volume of distribution (EMEA, 1997, 1998). The toxic effects are derived from cleavage of hyoscyamine into an alkamine and tropic acid by hepatic esterases (Glick and Glaubach, 1941, Wada *et al.*, 1991). Scopolamine undergoes oxidative demethylation and first-pass metabolism mediated by cytochrome P450 3A, so that only a small fraction (<5%) of the dose is excreted as the parent compound, however, the oxidation products have not yet been identified. Most atropine (60%) is excreted unchanged in the urine but for scopolamine urinary excretion is lower than 5% (Putcha *et al.* 1991, Ali-Melkkilä 1993, EMEA 1998, Renner *et al.* 2005, Chen *et al.* 2006).

4.3.2 Calystegines

Calystegines are potent inhibitors of β-glucosidase (Molyneux *et al.* 1993, Asano *et al.* 1995, 1997a, Asano 2000). Calystegines of the roots of *C. arvensis* and of the hedge bindweed (*C. sepium*) are potent inhibitors of β-glucosidase. They cause neurological dysfunction associated with glycosidase inhibition and lysosomal storage disorders (Watson *et al.* 2001).

In studies using mammalian tissues Asano *et al.* (1997a) found that calystegines B1 and C1 were potent competitive inhibitors of the bovine, human, and rat β-glucosidase activity and calystegine B2 was a strong competitive inhibitor of the α-galactosidase activity in liver. Calystegine B1 is also a potent competitive inhibitor of almond β-glucosidase and bovine liver β-galactosidase but it does not inhibit α-galactosidases. Calystegine B2 is also a potent competitive inhibitor of almond β-glucosidase and coffee bean α-galactosidase (Asano *et al.* 1995).

Mice fed convolvulus alone ate it readily but soon showed excitement and then depression, anorexia, hunched posture and death. Lesions were found in their stomachs and livers, with acute gastritis erosions and ulcerations, and hepatic necrosis. Mice offered bindweed as a choice ate it but had far milder effects. Human β-xylosidase was inhibited by all calystegines tested. Calystegines A3 and B2 selectively inhibited the rat liver β-glucosidase activity. Calystegine A3 is weaker than calystegine B2. Calystegine A5, had no activity against any glycosidases. Calystegine B3 (a 2-epimer of calystegine B2) has weak inhibitory activity, suggesting that the equatorially oriented -OH group at C2 is necessary for strong binding at the active site of glycosidases. Convolvine blocks M2 and M4 cholinergic muscarinic receptors (Mirzaev 1998).

Asano *et al.* (1997a) proposed that the potent inhibition of mammalian glucosidases *in vitro* raised concerns that calystegines were toxic to humans consuming large amounts of plants that contain them.

4.3.3 Minor TAs

There is very little information on the toxicity of individual TAs other than atropine and scopolamine. Pseudotropine causes intestinal contraction in the dog, followed by an inhibition of spontaneous contractions but tropine augments intestinal contractions (Hamlte 1931 quoted in Todd *et al.* 1995). Cuscohygrine suppresses an immune response in mice (Watson *et al.* 1983).

Anisodine produces four known metabolites (norscopine, scopine, hydroxyanisodine, *N*-oxide anisodine) as determined in rat plasma following ingestion (Chen *et al.* 2006).

5 IDENTIFICATION OF THE RELEVANT TA FROM FOODS AND ASSOCIATED PLANTS

Some assessment was made of toxic weed plants from outside the EU likely to contaminate foods imported into the EU. The demand for soya beans in the EU exceeds its cultivation and over 30 million tons are imported each year of which about 5% is used directly as an ingredient in human foods. Soya is frequently contaminated by seeds of toxic Solanum species often described as black nightshade (*Solanum nigrum* L.) but which is more likely eastern black nightshade (*Solanum ptycanthum*). The plant is common in cultivated fields and its fruits have been harvested with peas, snap beans and soybeans. Other solanums invasive and common in the USA and Asia are *Solanum viarum* and *Solanum torvum* (turkeyberry).

A report from Adamse and van Egmond (2010) clearly points to contamination with seeds from *D. stramonium* (Jimson weed or thorn apple) as the most common problem, however the authors indicate that seeds from other Datura spp. as well as berries of *Atropa belladonna* and seeds of *Hyoscyamus niger* have been reported as impurities in food.

TAs are found in the genera *Atropa*, *Brugmansia*, *Datura*, *Hyoscyamus*, *Mandragora* and *Scopolia* among the plants that are herbaceous and therefore more likely to act as weeds, and in many plants less likely to be found in Europe or in the vicinity of food crops, such as *Iochroma*, *Juanulloa*, and *Solandra*.

TAs (tropine, tropinone, cuscohygrine, hygrine) have been reported in *Convolvulus arvensis* and Convolvulus sepium, now known as *Calystegia sepium* (Goldmann *et al.* 1990).

Specialised Nutrition Europe (SNE) is a trade association representing the specialised nutrition industry across the European Union. SNE members are the national associations of 17 EU Member States and their companies produce foods for specific groups', including the foods specifically intended for infants and young children. An unreported survey by SNE carried out in 2014 confirmed data from the Dutch authorities that showed contamination risks in sorghum and millet. SNE has expressed its favour in setting up an Indicative Value of 250 µg/kg for the sum of hyoscyamine and scopolamine in sorghum, millet and buckwheat used to manufacture cereal- based foods for infants and young children (SNE 2014). http://www.anid.pt/attachments/article/45/alkaloids.pdf

6 IDENTIFICATION OF RELEVANT FOOD MATRICES USING THE FOLLOWING CRITERIA:

6.1 Foods associated with intoxications with TA

6.1.1 Foods associated with the TA containing weeds

Foods that have been associated with TA intoxications from weeds are cereal-based foods, particularly those intended for infants and young children and foods based on particular seeds, principally buckwheat, millet, poppy and sunflower. A second important category is vegetables, particularly green beans, both frozen and canned, which are occasionally contaminated with *Datura* fruit and with toxic species of *Solanum*.

6.1.2 Foods containing inherent TAs

Potato and potato products have had occasional historical poisoning associations but as acute symptoms are mild the incidence might be underreported.

6.2 Consumption levels

A summary of the consumption levels of the tropane-containing plants in European countries for which data was available via the FAO/WHO Chronic Individual Food Consumption Database – summary statistics (CIFOCOss) was obtained as published in 2015 (FAO/WHO 2015) and a summary of this is provided in Table 3. Data are provided in grams of plant per kg body weight per day (g/kg bw/day), although data have not been reported for some important countries. The data show that the highest levels of potato consumption to occur in children in Bulgaria (about 3.5 g/kg bw/day), in adolescents and children in the Czech Republic (2-3 g/kg bw/day), in adults and elderly people in Hungary and adults in Spain. There are no data for potato consumption in Belgium, France and the UK.

The highest levels of fresh tomato consumption are in Italy and Bulgaria (about 3 g/kg bw/day). The highest levels of brassica consumption are in the North Western European countries, and the highest consumption of green beans (included on account of more regular tropane contamination) are in France, Belgium, and Bulgaria.

Summaries of the consumption levels of the TA-containing plant foods and of foods that have been contaminated with TAs in 19 European countries (Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, United Kingdom) as reported by the EFSA Food Consumption Database are provided in Tables 4-11.

The tables generally show the number of subjects of the survey, the number of consumers of the products, and the average consumption in grams per day for all consumers. It should be noted that for some food categories the number of consumers are too low to allow a statistically robust analysis of the consumption. The EFSA consumer descriptions are for pregnant women, lactating mothers, infants, toddlers, other children, adolescents, adults, elderly, and very elderly. In most tables the consumer descriptions have a number prefix to enable sorting by age. Where there is more than one entry for a consumer description this is because data for more than one consumption survey is reported.

Table 4 shows the average consumption in grams per day for all subjects (i.e. for the general population) and all consumers (those individuals who actually consume the products) of a range of potatoes and potato products. Boiled potatoes are the major product in this group.

Table 5 shows a breakdown by country of the data supplied in Table 4 filtered to show only the higher consumers, i.e. the 95th percentile, those consuming more than the average quantity. The heaviest consumers are in northern, western and central Europe and tend to be adults and elderly.

Table 6 shows the average consumption in grams per day for infants, toddlers and other children of cereal based foods for infants and young children. High consumers are shown in bold. In this table (only) data are reported for all subjects including the non-consumers, in order to show the range of consumption in these vulnerable groups. There appears to be evidence of some targeting of subject groups in the data for Finland and Germany where in some cases several hundred subjects provide no consumers and in other cases there are a number of consumers. Consumption levels appear much higher in the data for toddlers in Spain.

Table 7 shows the average consumption in grams per day for high consumers of aubergines. There were approximately 4,000 consumers from 80,000 subjects, with an average intake of 1.5 grams/day. The highest consumers by some margin were in Romania and Italy.

Table 8 shows the average consumption in grams per day for high consumers of beans with and without pods. These data are provided on account of the occasional contamination of green beans and vegetable mixtures with Datura. From 7289 subjects there were approximately 2700 consumers of beans without pods. There were 7402 subjects and 2424 consumers of beans with pods with an average intake of 2.5 and 4.0 grams/day respectively. The highest consumers of beans without pods were across a range of ages in the UK where subjects typically consumed 7 x the European average. However these are likely to be beans cooked and canned in tomato sauce, in which contaminating vegetable material from Datura would be more easily identified. For beans with pods the high consumers were in the Netherlands, Romania and Spain (3 to 5 x the average).

Table 9 shows the average consumption in grams per day for all consumers of buckwheat products. In the case of Latvia, the presence of a high proportion of consumers is associated with a particularly high consumption level. These factors indicate that the product is popular in that country. In other countries elevated consumption levels are also related to a higher proportion of consumers.

Table 10 shows the average consumption in grams per day for all consumers of millet products. The proportion of consumers is low but notably higher in toddlers and other children in Finland. In most countries the consumption level does not greatly exceed the European average.

Table 11 shows the average consumption in grams per day for all consumers of poppy seeds. Consumption levels are highest in the Czech Republic, Hungary and Romania.

6.3 Availability of these foods on the EU market.

All of the foods described above are available on the EU market to degrees that vary with location. The foods that have been contaminated with TA-containing weeds are easily available across the EU. Many food plants with endogenous TAs (potatoes, aubergines, peppers, sweet potatoes) are widely available while others such as Cape gooseberry and goji berries have limited availability or market demand is current low.

7 Recommendations

The EFSA Opinion indicates contamination of a high proportion of cereal-based food for infants and young children, and a lesser contamination of a high proportion of cereal-based food intended for both toddlers and infants. Other contamination has been revealed in food and feed based on oilseeds, especially sunflower seeds, and in millet and related plants such as sorghum.

From reports under RASFF the greatest incidence of contamination has concerned *Datura* seeds in millet (mainly organic and mainly originating in Austria) and *Datura* fruit (berries) in processed bean-based vegetables. Frozen green beans have also been contaminated with *Solanum* and poppy seeds with *Hyoscyamus* (henbane).

Considering the widespread contamination of herbal products with pyrrolizidine alkaloids and a single incidence of contamination of tea prepared from marshmallow root with *Atropa belladonna* root there might be a requirement to survey herbal teas from areas with potential tropane-containing plant growth.

Regarding contamination of cereal crops with weeds, the major weeds currently contaminating food crops in the EU are *Datura stramonium* and *Convolvulus arvensis*. The TAs tropine, tropinone, cuscohygrine, hygrine have been reported in *Convolvulus arvensis* and *C. sepium* (*Calystegia sepium*). Whilst there are many reports of contamination by *Datura* tropanes there are no reports of calystegine contamination of foods in which they do not occur naturally. There appears to be widespread and sometimes heavy growth of *Convolvulus* in cereal fields, but consumer exposure to calystegines will be greater and more sustained through consumption of potato products.

Toxic weed plants from outside the EU likely to contaminate foods imported into the EU are *Solanum* species *S. ptycanthum, S. nigrum, S. viarum* and *S. torvum.* These plants are common in cultivated fields and have been harvested with peas, snap beans and soya beans. Other solanums invasive and common in the USA and Asia are *S. viarum* and *S. torvum* (turkeyberry).

The primary recommendations are for a focus on:

Processed cereal foods intended for babies, including a high proportion of organic products.

Processed cereal foods intended for infants and toddlers, including a high proportion of organic products.

Processed and unprocessed cereal foods for adults, including a high proportion of organic products, including some samples with poppy seeds and where identified some products containing millet.

Processed foods and flour based on buckwheat, including a high proportion of organic products, including some samples with poppy seeds and where identified some products containing millet.

Canned and frozen vegetables with a high proportion of green beans.

Potato products of all types should be analysed for calystegines with perhaps a recommendation to extend the survey in future to the field cereal products, especially if the extracts can be saved for a suitable time.

Regarding a focus on the geographic regions for sampling it can be assumed that in ignorance of the scale and location of the sampling that led to e.g. the RASFF reports it might be unwise to aim too strongly at the countries where problems have been reported previously.

The review has highlighted the emerging potential for food contamination from non-food weed plants that are encroaching on field crops in Europe. *Convolvulus* species (bindweeds) present a particular problem that should be addressed in future projects.

8 REFERENCES

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Annex 1. Literature review on Tropane Alkaloids in Food

Table 28. RASFF notifications and/or alerts for *Datura*, *Solanum*, and *Hyoscyamus*.

Type	Date of case	Country	Product Category	Food	Origin	Contaminant	µg/kg		%
							atropine	scopolamine	
alert	31/03/2015	Germany	cereals and bakery products	millet balls	Hungary	atropine	4.1 to 384	2.0 to 388	
alert	30/03/2015	Austria	cereals and bakery products	millet honey poppies	Germany	atropine	26	11	
alert	30/03/2015	Austria	cereals and bakery products	organic millet	Austria	atropine	30	24	
alert	20/03/2015	Austria	cereals and bakery products	millet dumplings	Hungary	atropine	0.30	0.36	
alert	20/03/2015	Austria	cereals and bakery products	millet dumplings	Hungary	atropine	0.48	0.53	
alert	20/02/2015	Austria	cereals and bakery products	polenta	Germany	atropine	0.06	0.03	
alert	20/02/2015	Germany	cereals and bakery products	porridge	Germany	atropine	156.2 to 207.5	31.3	
alert	18/12/2014	Germany	food supplements, fortified foods	millet	Hungary	atropine	36.6		
alert	11/12/2014	Germany	food supplements, fortified foods	millet	Germany	atropine	3.73 to 31.9		
alert	04/12/2014	Germany	cereals and bakery products	millet	Hungary	atropine	46	25	
alert	21/11/2014	Germany	food supplements, fortified foods	baby food	Austria	atropine	12.1		
alert	13/06/2013	Slovenia	cereals and bakery products	buckwheat	Slovakia	atropine	18	5.5	
alert	22/05/2013	Slovenia	cereals and bakery products	buckwheat	Czech Republic	atropine	14	11	
information	08/06/2012	Slovenia	cereals and bakery products	buckwheat	Slovenia	atropine	20	6.1	
information	30/04/2009	Slovenia	cereals and bakery products	buckwheat	Hungary	atropine	110	48	
information	03/07/2006	Slovenia	cereals and bakery products	buckwheat	Ukraine	atropine	37	47	
alert	03/07/2006	Slovenia	cereals and bakery products	buckwheat	Czech Republic	atropine	35	65	
alert	28/10/1994	UK	cocoa, coffee and tea	burdock root tea	UK	atropine			
alert	17/05/2013	Finland	fruits and vegetables	veg.-bean-seed mix	Spain	Datura fruit			
alert	05/09/2007	Austria	fruits and vegetables	green beans canned	Hungary	Datura fruit			
alert	27/11/2006	Austria	fruits and vegetables	green beans canned	Hungary	Datura fruit			
alert	11/12/2014	Germany	food supplements, fortified foods	millet baby food	Germany	Datura seeds	3.7 to 32		
information	02/10/2007	Spain	fruits and vegetables	vegetable stir-fry	Spain	Datura seeds			
alert	27/11/2006	Austria	cereals and bakery products	millet	Austria/Hungary	Datura seeds			13
information	20/11/2006	Austria	cereals and bakery products	millet brown	Austria	Datura seeds			
information	08/11/2006	Austria	cereals and bakery products	millet	Austria	Datura seeds			
alert	24/10/2006	Austria	cereals and bakery products	millet	Austria	Datura seeds			
information	10/10/2006	Austria	cereals and bakery products	millet	Austria	Datura seeds			

Table 1. (continued) RASFF notifications and/or alerts for *Datura*, *Solanum*, and *Hyoscyamus*.

Type	Date of case	Country	Product Category	Food	Origin	Contaminant	%
alert	06/05/2008	Czech Republic	nuts, nut products and seeds	poppy seeds	Czech Republic	henbane seeds	0.13
alert	13/04/2007	Slovakia	nuts, nut products and seeds	poppy seeds	Czech Republic	henbane seeds	0.24
alert	10/04/2007	Slovakia	nuts, nut products and seeds	poppy seeds	Czech Republic	henbane seeds	0.42
border rejection	06/03/2015	Portugal	food supplement	ingredient	Portugal	*Solanum nigrum*	
alert	07/06/2013	Germany	fruits and vegetables	green beans canned	Netherlands	*Solanum nigrum*	
alert	18/02/2004	France	fruits and vegetables	green beans frozen	France	*Solanum nigrum*	
alert	06/11/1985	Germany	fruits and vegetables	green beans canned	Denmark	*Solanum nigrum*	
alert	13/10/1982	Italy	fruits and vegetables	green beans frozen	Italy	*Solanum nigrum*	

Table 29. Food plants in the Solanaceae family that contain TA.

Species	**Name**	**Consumption**
Capsicum annuum	Sweet pepper, chili pepper, paprika	high
Solanum lycopersicum	Tomato	high
Solanum melongena	Aubergine or /Eggplant	high
Solanum tuberosum	Potato	high
Capsicum chinensis	Various hot peppers	low
Capsicum frutenscens.	Cayenne pepper	low
Physalis ixocarpa	Tomatillo	low
Physalis peruviana	Cape Gooseberry	low
Solanum betaceum	Tamarillo	low
Solanum muricatum	Pepino	low
Solanum quitoense	Naranjilla	low

Table 30. Consumption levels of the tropane-containing plants in European countries for which data was available from the FAO/WHO Chronic Individual Food Consumption Database

Country	Age class	Potato	Tomato	Sweet potato	Brassica	Green beans
Belgium	Adolescents	0.00	0.53	b	0.011	0.054
Belgium	Adults	0.01	0.50	b	0.024	0.071
Belgium	Elderly	0.00	0.41	b	0.018	0.150
Belgium	Other children	b	0.66	b	0.006	b
Belgium	Toddlers	b	0.65	b	0.043	b
Belgium	Very elderly	b	b	b	0.027	0.116
Bulgaria	Other children	3.54	3.20	b	b	0.124
Bulgaria	Toddlers	3.87	3.07	b	b	0.144
Bulgaria	Infants	1.64	0.80	b	b	0.030
Czech Republic	Adolescents	2.08	0.37	b	0.009	b
Czech Republic	Adults	1.17	0.25	b	0.010	b
Czech Republic	Other children	2.87	0.35	b	0.018	b
Denmark	Adolescents	b	0.71	b	0.016	b
Denmark	Adults	b	0.54	b	0.021	b
Denmark	Elderly	b	0.45	b	0.020	b
Denmark	Other children	b	0.94	b	0.018	b
Denmark	Very elderly	b	0.44	b	0.023	b
Finland	Adults	0.24	0.43	0.004	0.020	b
Finland	Elderly	0.34	0.37	0.003	0.015	b
Finland	Other children	0.12	0.50	b	0.027	b
Finland	Other children	b	b	b	0.029	b
Finland	Toddlers	0.04	0.37	b	0.001	b
France	Adolescents	b	0.37	b	b	0.247
France	Adolescents	b	b	0.004	b	b
France	Adults	b	0.38	b	b	0.247
France	Adults	b		0.002	b	b
France	Elderly	b	0.39	b	b	0.281
France	Other children	b	0.67	b	b	b
France	Other children	b		0.002	b	0.546
France	Very elderly	b	0.38	b	b	0.324
Greece	Other children	b	0.44	b	b	b
Hungary	Adults	1.53	0.14	b	0.000	b
Hungary	Elderly	1.43	0.14	b	b	b
Hungary	Very elderly	1.68	0.13	b	b	b
Ireland	Adults	0.07	0.52	0.005	0.001	b

Table 3 (Continued) Consumption levels of the tropane-containing plants in European countries for which data was available from the FAO/WHO Chronic Individual Food Consumption Database

Country	Age class	Potato	Tomato	Sweet potato	Brassica	Green beans
Italy	Adolescents	b	1.55	b	b	0.057
Italy	Adults	0.00	1.16	0.001	b	0.044
Italy	Elderly	0.00	1.04	0.001	b	0.056
Italy	Other children	b	2.65	b	b	0.076
Italy	Toddlers	b	2.60	b	b	0.119
Italy	Infants	b	0.08	b	b	b
Italy	Very elderly	b	0.98	b	b	0.040
Latvia	Adolescents	b	b	b	0.001	0.020
Latvia	Adults	b	b	b	0.001	0.005
Netherlands	Adults	b	b	b	0.076	b
Netherlands	Other children	b	b	b	0.043	b
Netherlands	Toddlers	b	b	b	0.089	b
Spain	Adolescents	1.34	0.63	0.002	b	b
Spain	Adolescents	b	0.45	b	b	b
Spain	Adolescents	b	0.32	b	b	b
Spain	Adults	0.81	0.91	0.001	b	0.004
Spain	Adults	1.14	0.94	b	b	0.002
Spain	Other children	b	0.76	0.026	b	b
Spain	Other children	b	0.30	b	b	b
Spain	Toddlers	b	0.27	0.108	b	b
Sweden	Adolescents	0.00	0.15	b	0.000	0.004
Sweden	Adults	0.00	0.37	b	0.003	0.005
Sweden	Other children	0.01	b	b	0.002	0.009
United Kingdom	Adults	0.23	0.39	0.004	0.010	b

EFSA Tables

Table 31. EFSA Average potato and potato product consumption for all countries (grams/day).

Item	No. Subjects	No. Consumers	All Subjects	All Consumers
Potato boiled	80697	34578	30.7	45.8
Main-crop potatoes	725965	92210	8.1	32.0
Potatoes and potato products	80697	19890	20.4	28.2
Potato fried	80697	6813	4.5	9.8
New potatoes	80697	2617	2.4	8.1
Potato baked	80697	4995	2.6	4.8
Potato crisps	80697	11306	2.1	2.4
Mashed potato powder	80697	4232	0.8	2.2
Potato croquettes	80697	755	0.3	0.8

Table 32. EFSA Consumption data for potatoes and potato products

Product	Consumer	No. Subjects	No. Consumers	g/day
Potato boiled	**High consumers**			
Belgium	Elderly, Very elderly	1215	1002	113
Ireland	Adults, Elderly, Very elderly	2458	2140	114
Latvia	Pregnant women, Adolescents, Adults	2726	1991	83
Netherlands	Elderly, Very elderly	912	660	77
Sweden	Other children, Adolescents	2491	2077	66
Sweden	Adults, Elderly, Very elderly	3007	2386	100
Main-crop potatoes	**High consumers**			
Czech Republic	4. Adolescents	298	223	92
Czech Republic	3. Other children	389	304	72
Czech Republic	5. Adults	1666	1222	87
Hungary	7. Very elderly	80	73	114
Hungary	6. Elderly	206	179	104
Hungary	5. Adults	1074	912	108
Romania	7. Very elderly	45	43	75
Romania	6. Elderly	83	80	88
Romania	5. Adults	1254	1151	75
Potatoes and pot. products	**High consumers**			
Belgium	3. Other children	625	535	50
Belgium	2. Toddlers	36	27	45
Denmark	4. Adolescents	377	351	70
Denmark	4. Adolescents	479	453	84
Denmark	5. Adults	1739	1683	89
Denmark	5. Adults	2822	2750	105
Denmark	6. Elderly	274	270	128
Denmark	6. Elderly	309	306	143
Denmark	1. Infants	826	764	39
Denmark	3. Other children	298	284	53
Denmark	3. Other children	490	470	58
Denmark	2. Toddlers	917	842	24
Denmark	7. Very elderly	12	12	89
Denmark	7. Very elderly	20	20	175

Table 33. EFSA Consumption data for cereal-based food for infants and young children

Country	Consumer	No. Subjects	No. Consumers	g/day
Austria	3. Other children	128	0	0.0
Belgium	2. Toddlers	36	0	0.0
Belgium	3. Other children	625	1	0.0
Bulgaria	1. Infants	859	9	0.2
Bulgaria	2. Toddlers	428	2	0.2
Bulgaria	3. Other children	433	0	0.0
Czech Republic	3. Other children	389	4	0.2
Denmark	1. Infants	826	0	0.0
Denmark	2. Toddlers	917	0	0.0
Denmark	3. Other children	490	0	0.0
Denmark	3. Other children	298	0	0.0
Finland	1. Infants	500	0	0.0
Finland	2. Toddlers	500	0	0.0
Finland	**2. Toddlers**	**497**	**72**	**7.3**
Finland	3. Other children	750	0	0.0
Finland	3. Other children	250	0	0.0
Finland	3. Other children	933	7	0.4
France	3. Other children	482	0	0.0
Germany	1. Infants	159	0	0.0
Germany	2. Toddlers	348	0	0.0
Germany	**2. Toddlers**	**261**	**15**	**2.9**
Germany	3. Other children	293	0	0.0
Germany	3. Other children	835	0	0.0
Germany	3. Other children	660	3	0.7
Greece	3. Other children	838	0	0.0
Italy	1. Infants	16	0	0.0
Italy	2. Toddlers	36	0	0.0
Italy	3. Other children	193	0	0.0
Latvia	3. Other children	187	0	0.0
Netherlands	**2. Toddlers**	**322**	**52**	**2.7**
Netherlands	3. Other children	447	0	0.0
Netherlands	3. Other children	957	42	0.8
Spain	**2. Toddlers**	**17**	**8**	**41.6**
Spain	3. Other children	399	0	0.0
Spain	**3. Other children**	**156**	**7**	**2.4**
Sweden	**3. Other children**	**1473**	**33**	**4.7**
UK	1. Infants	1369	45	0.3
UK	2. Toddlers	185	4	0.2
UK	2. Toddlers	1314	47	0.4
UK	3. Other children	651	2	0.0
Sum		80697	357	0.6
Average for all consumers				3.2

Table 34. EFSA Consumption data for aubergines

Country	Consumer	No. Subjects	No. Consumers	g/day
Bulgaria	2. Toddlers	428	27	2.8
Bulgaria	3. Other children	433	37	6.2
France	5. Adults	2276	588	2.4
France	6. Elderly	264	61	2.8
Greece	0. Lactating women	65	10	4.1
Italy	2. Toddlers	36	4	2.2
Italy	3. Other children	193	26	6.5
Italy	4. Adolescents	247	34	9.2
Italy	5. Adults	2313	427	9.9
Italy	6. Elderly	290	43	8.9
Italy	7. Very elderly	228	33	7.6
Romania	5. Adults	1254	542	11.8
Romania	6. Elderly	83	45	15.1
Romania	7. Very elderly	45	24	18.6
Spain	5. Adults	410	38	3.4
Spain	5. Adults	981	69	2.2
sum		80697	3971	
Average for consumers				1.5

Table 35a. EFSA Consumption data for high consumers of beans (*Phaseolus vulgaris*)

Country	Consumer	No. Subjects	No. Consumers	g/day
Romania	6. Elderly	83	40	8.1
Romania	7. Very elderly	45	22	9.0
Spain	3. Other children	399	54	9.1
Spain	4. Adolescents	651	94	12.5
United Kingdom	2. Toddlers	185	94	15.7
United Kingdom	2. Toddlers	1314	483	8.9
United Kingdom	3. Other children	651	284	16.1
United Kingdom	4. Adolescents	666	234	17.6
United Kingdom	5. Adults	1266	417	17.6
United Kingdom	5. Adults	1724	865	16.7
United Kingdom	6. Elderly	166	47	14.5
United Kingdom	7. Very elderly	139	31	8.6
Sum		7289	2665	
Average for all consumers				2.5

Table 8b EFSA Consumption data for high consumers high consumers of beans (*Phaseolus vulgaris*) with pods

Country	Consumer	No. Subjects	No. Consumers	g/day
Hungary	6. Elderly	206	29	8.8
Netherlands	4. Adolescents	1142	211	9.6
Netherlands	5. Adults	2057	385	11.6
Netherlands	6. Elderly	173	42	18.8
Netherlands	6. Elderly	289	75	20.1
Netherlands	7. Very elderly	450	134	23.4
Romania	5. Adults	1254	893	14.9
Romania	6. Elderly	83	61	17.1
Romania	7. Very elderly	45	31	16.9
Spain	2. Toddlers	17	4	17.1
Spain	4. Adolescents	209	39	10.5
Spain	4. Adolescents	86	33	14.9
Spain	5. Adults	410	94	11.1
Spain	5. Adults	981	393	16.0
Sum		7402	2424	
Average for all consumers				4.0

Table 36. EFSA Consumption data for all consumers of buckwheat

Country	Consumer	Product	No. Subjects	No. Consumers	g/day
Germany	2. Toddlers	Bread	261	2	0.596
Denmark	5. Adults	Flour	1739	7	0.007
Finland	5. Adults	Flour	1575	9	0.181
Finland	6. Elderly	Flour	463	10	0.970
Germany	2. Toddlers	Flour	261	1	0.024
Germany	3. Other children	Flour	835	1	0.003
Germany	3. Other children	Flour	660	3	0.012
Germany	5. Adults	Flour	10419	1	0.000
Belgium	5. Adults	Grain	1292	1	0.024
Belgium	7. Very elderly	Grain	704	1	0.122
Finland	1. Infants	Grain	500	2	0.016
Finland	2. Toddlers	Grain	500	16	0.571
Finland	3. Other children	Grain	750	35	0.227
Finland	4. Adolescents	Grain	306	1	0.032
Germany	2. Toddlers	Grain	348	1	0.000
Germany	3. Other children	Grain	293	1	0.005
Germany	3. Other children	Grain	660	1	0.002
Germany	3. Other children	Grain	835	1	0.024
Germany	5. Adults	Grain	10419	2	0.007
Germany	6. Elderly	Grain	2006	1	0.043
Italy	4. Adolescents	Grain	247	4	0.270
Italy	5. Adults	Grain	2313	14	0.100
Italy	6. Elderly	Grain	290	4	0.224
Italy	7. Very elderly	Grain	228	1	0.073
Netherlands	6. Elderly	Grain	289	2	0.007
Sweden	3. Other children	Grain	1473	1	0.014
UK	1. Infants	Grain	1369	1	0.001
UK	2. Toddlers	Grain	185	1	0.095
UK	2. Toddlers	Grain	1314	7	0.068
UK	3. Other children	Grain	651	2	0.031
Austria	5. Adults	Groats	308	3	0.386
Ireland	5. Adults	Groats	1274	1	0.005
Latvia	**3. Other children**	**Groats**	**187**	**31**	**10.896**
Latvia	**4. Adolescents**	**Groats**	**453**	**52**	**9.575**
Latvia	**5. Adults**	**Groats**	**1271**	**154**	**10.250**
Latvia	**Pregnant women**	**Groats**	**1002**	**178**	**16.205**
Ireland	5. Adults	Milling products	958	1	0.008
Finland	2. Toddlers	Milling products	497	11	0.405
Finland	3. Other children	Milling products	933	31	0.204

Table 37. EFSA Consumption data for all consumers of millet

Country	**Consumer**	**Product**	**No. Subjects**	**No. Consumers**	**g/day**
Belgium	4. Adolescents	Grain	576	1	0.069
Belgium	6. Elderly	Grain	511	1	0.093
Finland	2. Toddlers	Grain	500	18	0.179
Finland	3. Other children	Grain	750	61	0.201
Finland	6. Elderly	Grain	413	1	0.097
Germany	1. Infants	Grain	159	5	0.254
Germany	2. Toddlers	Grain	261	3	0.074
Germany	2. Toddlers	Grain	348	14	0.168
Germany	3. Other children	Grain	293	10	0.057
Germany	3. Other children	Grain	660	7	0.069
Germany	3. Other children	Grain	835	3	0.166
Germany	4. Adolescents	Grain	393	1	0.068
Germany	6. Elderly	Grain	2006	2	0.066
Hungary	7. Very elderly	Grain	80	1	0.063
Italy	6. Elderly	Grain	290	1	0.092
Sum			**80697**	**154**	
Austria	5. Adults	Groats	308	2	0.263
Latvia	3. Other children	Groats	187	1	0.535
Latvia	0. Pregnant women	Groats	1002	9	0.773
Sweden	5. Adults	Groats	1210	1	0.017
Sum			**80697**	**13**	
Austria	5. Adults	Flakes	308	4	0.195
Austria	7. Very elderly	Flakes	25	2	0.600
Denmark	2. Toddlers	Flakes	917	7	0.028
Germany	1. Infants	Flakes	159	7	0.329
Germany	2. Toddlers	Flakes	261	2	0.026
Germany	5. Adults	Flakes	10419	6	0.015
Germany	6. Elderly	Flakes	2006	3	0.007
Sum			**79871**	**31**	
Finland	2. Toddlers	Flour	497	20	0.170
Finland	3. Other children	Flour	933	31	0.040
Finland	5. Adults	Flour	1575	1	0.018
Finland	6. Elderly	Flour	463	3	0.100
Germany	2. Toddlers	Flour	348	1	0.003
Germany	3. Other children	Flour	835	1	0.002
Germany	3. Other children	Flour	660	1	0.008
Germany	3. Other children	Flour	293	1	0.014
Sum			**80697**	**59**	
Average for all consumers					0.045

Table 38. EFSA Consumption data for all consumers of poppy seed

Country	**Consumer**	**No. Subjects**	**No. Consumers**	**g/day**
Austria	3. Other children	128	3	0.30
Austria	4. Adolescents	237	5	0.19
Austria	5. Adults	308	1	0.02
Czech Republic	3. Other children	389	22	0.39
Czech Republic	4. Adolescents	298	11	0.41
Czech Republic	5. Adults	1666	40	0.25
Germany	2. Toddlers	261	1	0.03
Germany	3. Other children	293	1	0.01
Germany	3. Other children	660	2	0.02
Germany	3. Other children	835	6	0.03
Germany	5. Adults	10419	3	0.00
Hungary	5. Adults	1074	57	0.46
Hungary	6. Elderly	206	18	0.60
Hungary	7. Very elderly	80	3	0.28
Ireland	5. Adults	1274	1	0.00
Ireland	6. Elderly	149	1	0.00
Romania	5. Adults	1254	37	0.12
Romania	6. Elderly	83	1	0.03
United Kingdom	3. Other children	651	4	0.00
United Kingdom	5. Adults	1266	6	0.01
United Kingdom	6. Elderly	166	1	0.02
Sum		80697	224	
Average for all consumers				**0.15**

APPENDIX

Strategy for identification and characterisation of relevant tropane alkaloids and food products

The table below lists the suggested search topics and combinations for searching.

Provisional indications are that so few results are obtained from including "calystegine" as a search term that an independent single search for this topic will be the best approach.

	Search 1 returns records on toxic effects of tropane alkaloids
# 16	# 15 AND # 2
# 15	# 12 OR # 13 OR # 14
# 14	TOPIC: (intox*)
# 13	TOPIC: (food* SAME poison*)
# 12	TOPIC: (tox* OR harmful* OR adverse*)
	Search 2 returns records on occurrence of tropane alkaloids in food, feed, and plants
# 11	# 10 AND # 2
# 10	# 9 OR # 8 OR # 7 OR # 6 OR # 5 OR # 4 OR # 3
# 9	TOPIC: (weed OR weeds OR cereal* OR pea* solanac OR brassica*)
# 8	TOPIC: (plant* SAME deriv* SAME contam*)
# 7	TOPIC: ((plant or plants) NEAR/2 assess*)
# 6	TOPIC: (risk* NEAR/2 assess*)
# 5	TOPIC: (provenance OR source*)
# 4	TOPIC: (natural* NEAR/2 occur*)
# 3	TOPIC: (food* OR feed*)
# 2	TOPIC: (Tropane OR Tropinone OR Tropine OR Pseudotropine OR Scopine OR Scopoline OR Tigloytropine OR Tigloidine OR Cusohygrine OR *Tropanyl phenylacetate* OR Apoatropine OR Homatropine OR Convolidine OR Phygrine OR Apohyoscine OR Aposcopolamine OR Littorine OR Convoline OR Atroscine OR Convolamine OR *Beta-hydroxyhyoscyamine OR Hydroxyhyoscyamine OR Anisodine OR Belladonine OR Atropa OR Brugmansia OR Datura OR Duboisia OR Erycibe OR Hyoscyamus OR Latua OR Lycium OR MandragORa OR Nicotiana OR Physalis OR Scopolia OR Brassica OR Calystegia OR Convolvulus)
# 1	TOPIC: (Calystegine)

Searching in multiple databases on 'Web of Science' did not include use of the Chemical Abstracts Registry (CAS) Number. The searchs can be repeated using the ‘CAB Abstracts® and Global Health®’ databases, using the CAS numbers to replace the chemical names in # 2.

Annex 2. Standard Operating Procedure for Determination of Tropane Alkaloids

Standard Operation Protocol for LC-MS/MS analysis of TAs in cereals, cereal products (herbal) dry tea, (herbal) tea infusions and stir-fry vegetable products

SOP for EFSA project Occurrence of TAs in food

Patrick Mulder & Patricia López Sánchez, February 2016, version g

Introduction

- This document describes the validated SOP for the LC-MS/MS analysis of TAs in cereals and cereal products and (herbal) dry teas in the range of 0.5 to 50 μg/kg.
- This document describes the SOP for the LC-MS/MS analysis of TAs in frozen legume products in the range of 0.5 to 50 μg/kg.

TAs are extracted from solid matrices with an acidic aqueous/methanolic solution. The extracts are purified and concentrated by means of solid phase extraction on a polymeric strong cation exchange cartridge. Tea infusions are purified and concentrated by means of solid phase extraction on a polymeric strong cation exchange cartridge. The purified extract is analysed by LC-MS/MS.

Part A: Standards, solutions and equipment

1. <u>Mixed standard solutions of TAs (10 and 100 ng/ml) in methanol</u>

1.1 Tropane alkaloids included in the method are:

- Small TAs (7 compounds): 6-hydroxytropinone, nortropinone, pseudotropine, scopine, scopoline, tropine, tropinone.
- Regular TAs (17 compounds): acetylscopolamine, anisodamine, anisodine, apoatropine, aposcopolamine, atropine, convolamine, convolidine, convolvine, fillalbin, homatropine, α-hydroxymethylatropine, littorine, noratropine, norscopolamine, phenylacetoxytropane, scopolamine.

These TAs are available as stock solutions (100 μg/ml) in methanol.

1.2 TA mixed standard solution (4 μg/ml) in methanol.

- Take 200 μl of each TA stock solution (100 μg/ml), add 200 μl methanol and mix.

1.4 TA mixed standard spiking solution (1000 ng/ml) in methanol.

- Take 4.0 ml of mixed standard solution (4 μg/ml), add 12.0 ml of methanol and mix.

1.5 TA mixed standard spiking solution (100 ng/ml) in methanol.

- Take 0.25 ml of TA mixed standard spiking solution (4 μg/ml), add 9.75 ml of methanol and mix.

All solutions are stored at -20°C in the dark for the duration of the project.

2. <u>Mixed internal standard solution of atropine-d3 and scopolamine-d3 (1000 ng/ml) in methanol</u>

2.1 Internal standards included in the method are:

- Atropine-d3 and scopolamine-d3.

These TAs are available as stock solutions (100 µg/ml) in methanol.

2.2 IS mixed spiking solution (1000 ng/ml) in methanol.

- Take 500 µl of each stock solution (100 µg/ml) and mix with 49 ml methanol.

The solution is stored at -20ºC in the dark for the duration of the project.

3. Extraction and SPE washing solutions

3.1 Solvents and chemicals.

3.1.1 Formic acid, glacial.

3.1.2 Methanol, UPLC quality.

3.1.3 Acetonitrile, UPLC quality.

3.1.4 Conc. ammonia (25%) in water.

3.1.5 Ammonium carbonate, anhydrous, p.a. quality.

3.1.6 Water, purified by a Milli-Q purification system, having a minimal resistance of 18.2 MΩ/cm.

3.2 Solutions

3.2.1 Extraction solvent: methanol/water/formic acid solution (75/25/0.4%).

- Mix 4 ml formic acid (3.1.1) with 750 ml methanol (3.1.2) and 250 ml water (3.1.6).

3.2.2 1% formic acid solution in water.

- Mix 1 ml formic acid (3.1.1) with 100 ml of water (3.1.6).

3.2.3 Solvent for sample extracts: 10% methanol in water.

- Mix 90 ml of water (3.1.6) with 10 ml of methanol (3.1.2).

3.2.4. 0.5% ammonia in methanol.

- Mix 2 ml ammonia 25% (3.1.4) with 100 ml methanol (3.1.2).

Note: For Strata X-C prepare 1% ammonia in methanol by mixing 4 ml ammonia 25% (3.1.4) with 100 ml methanol (3.1.2).
Ammoniated methanol (prepared by bubbling ammonia gas through a solution of methanol) may be used as an alternative.

3.2.5 Mobile phase A for LC-MS/MS: 10 mM ammonium carbonate pH 10.

- Dissolve 0.96 g ammonium carbonate (3.1.5) in 1 l water (3.1.6) and adjust to pH 10.0 with ammonia 25% (3.1.4).

3.2.6 Mobile phase B for LC-MS/MS: acetonitrile (3.1.3).

All solutions are stored at room temperature and can be used for 3 months.

3.2.7 Working standard solution of 10 ng/ml
Take 10 µL of mixed TA standard solution of 1000 ng/ml (1.4) and 10 µL of IS mixed spiking solution of 1000 ng/ml (2.2) and mix with 980 µL water (3.1.6).

This solution should be prepared every new day of analysis.

4. Equipment

4.1 Balance with a minimal weight range of 0 until 500 gram and with a precision of 0.01 g (e.g. Mettler Toledo GP503-S).
4.2 Vortex mixer (e.g. IKA Vortex 3).
4.3 Overhead shaker (e.g. Heidolph REAX 2).
4.4 Centrifuge suitable for 12 and 50 ml centrifuge tubes (e.g. MSE Falcon 6/300R).
4.5 Polypropylene tubes of 50 ml with screw cap (e.g. Greiner 210261).
4.6 Polypropylene tubes of 10 ml with screw or plug cap (e.g. Greiner 163270).
4.7 Dispenser 5-50 ml (e.g. Dispensette 4701361).
4.8 Pipettes for various solvent volumes, including a positive displacement pipet for organic solvents (e.g. Pipetman).
4.9 SPE Strong Cation Exchange cartridges: Oasis MCX 150 mg/6 cc, 30 µm (Waters 186000256) or Strata-X-C 200 mg/6 ml, 33 µm (Phenomenex 8B-S029-FCH).
4.10 SPE vacuum manifold (e.g. Alltech).
4.11 Vacuum pump (e.g. KNF Lab Laboport UN842.3FTP).
4.12 Evaporator with nitrogen flow, suitable for 12 ml tubes (e.g. Zymark TurboVap LV).
4.13 Filtervial PTFE 0.45 µm, 500 µl (e.g. Whatman UN203NPUORG).
4.14 Compressor for filtervials 6 positions (e.g. Whatman CR0000006).

Part B: Extraction and clean-up

5. Sample pre-treatment and test sample size for cereals, cereal products, (herbal) dry tea and stir-fry products

- The test sample size is 4 g for plain flours, breakfast cereals, bread, cookies and (herbal)dry tea.
- Laboratory samples of bread and stir-fry products stored at -20ºC are thawed before processing.
- Transfer the test samples to PP tubes of 50 ml (4.5).
- For the stir-fry vegetable products two test samples of 4 g are taken.

6. Extraction and sample preparation procedure for cereals, cereal products and (herbal) dry tea

6.1 MMS, MMRS and QC samples.
For preparation of MMS and MMRS samples a blank material is used of the same matrix type (i.e. buckwheat, millet, breakfast cereal, bread, cookies, green tea).

- 8 Matrix matched standards (MMS): spike the blank material (provided by RIKILT) with 0 - 50 ng/g TA std mix with a positive displacement pipet (4.8). See Table:

MMS (ng/g)	TA std mix 100 ng/ml (1.5) (µL)	TA std mix 1000 ng/ml (1.4) (µL)
0	-	-
0.5	20	-
1	40	-
2.5	100	-
5	200	-

10	-	40
25	-	100
50	-	200

- Mix samples by vortexing and let stand for 30 min.

- 1 Matrix matched recovery sample (MMRS): blank sample that will be spiked after clean up with TA std mix at 10 ng/g (see 6.4). For this sample MMS 0 ng/g can be used (additional 10 ml of blank extract, see 7.2)
- 2 QC samples (of same matrix type, provided by RIKILT): one sample not spiked and one spiked with TA std mix at 10 ng/ml.

The QC samples are provided by RIKILT as a pre-spiked set, containing 4 g amounts in 50 ml PP tubes.

6.2 Extraction.

- To all samples: add 10 ng/g IS (= 40 µl of IS solution 1000 ng/ml (2.1) with a positive displacement pipet (4.8). Mix sample by vortexing and let stand for 30 min.
- Add 40 ml of extraction solution (3.2.1) to the tubes by using a dispenser (4.7).
- Extract for 30 min on a rotary tumbler (overhead shaker) (4.3).
- Centrifuge for 15 min at 3500 rpm.
- Take 10 ml extract for SPE purification.

NOTE: At this point the samples can be stored overnight in the refrigerator.

6.3 Solid phase extraction

6.3.1 For cereal based products

- SPE cartridge: OASIS MCX 150 mg/6cc or Strata X-C, 200 mg/6 ml (4.9).
- Condition cartridge with 6 ml methanol (3.1.2).
- Equilibrate cartridge with 6 ml of 1% formic acid solution (3.2.2).
- Apply **10 ml of extract** to the cartridge.
- Wash cartridge with 6 ml of 0.4% formic acid solution in methanol/water 75/25 (3.2.1).
- Dry cartridge under reduced pressure.
- Elute cartridge with 6 ml of 0.5% ammonia in methanol (3.2.4) and collect eluates in 10 ml PP tubes with screw cap (4.6). (for Strata X-C use 6 ml of 1% ammonia in methanol). (alternatively ammoniated methanol may be used as well)
- Evaporate to dryness under a nitrogen atmosphere in a water bath of 50°C using a Turbovap (4.13).

NOTE: At this point the tubes containing the dried extracts can be stored in the freezer until further processing and analysis.

6.3.2 For dry (herbal)teas

- SPE cartridge: OASIS MCX 150 mg/6cc or Strata X-C, 200 mg/6 ml (4.9).
- Condition cartridge with 6 ml methanol (3.1.2).
- Equilibrate cartridge with 6 ml of 1% formic acid solution (3.2.2).
- Apply **5 ml of extract** to the cartridge.
- Wash cartridge with 6 ml of 0.4% formic acid solution in methanol/water 75/25 (3.2.1).
- Dry cartridge under reduced pressure.

- Elute cartridge with 6 ml of 0.5% ammonia in methanol (3.2.4) and collect eluates in 10 ml PP tubes with screw cap (4.6). (for Strata X-C use 6 ml of 1% ammonia in methanol). (alternatively ammoniated methanol may be used as well)
- Evaporate to dryness under a nitrogen atmosphere in a water bath of 50ºC using a Turbovap (4.13).

6.4 Preparation of extracts for LC-MS/MS analysis.
6.4.1 For cereal based products

- Dissolve the dry residue in 500 µl of sample extract solvent (3.2.3) and vortex for 15 s.

NOTE: To the MMRS extract, 10 µl of a TA std mix solution (1000 ng/ml TA std mix) in methanol is added and 490 µl water (3.1.6).

- Transfer extract to 500 µl filtervials (4.14) and close.

NOTE: At this point vials containing the final extracts can be stored in the freezer until analysis.

6.4.2 For dry (herbal)teas

- Dissolve the dry residue in 500 µl of sample extract solvent (3.2.3) and vortex for 15 s.

NOTE: To the MMRS extract, 5 µl of a TA std mix solution (1000 ng/ml TA std mix) in methanol is added and 495 µl water (3.1.6).

- Transfer extract to 500 µl filtervials (4.14) and close.

NOTE: At this point vials containing the final extracts can be stored in the freezer until analysis.

7. <u>Extraction and sample preparation procedure for stir-fry vegetable products</u>

For each sample two test samples are extracted:

- Blank test sample.
- Test sample spiked at 10 ng/g: add 40 µL of TA std mix at 1000 ng/ml with a positive displacement pipet (4.8). Mix sample by vortexing and let stand for 10 min.

Samples are further processed as described under 6. Extraction and sample preparation procedure for cereals, cereal products and (herbal dry tea).

Part C: LC-MS/MS analysis

8. <u>LC-MS/MS analysis.</u>

8.1 Instrumentation.
A Waters Xevo TQ-S tandem mass spectrometer, operated in positive electrospray mode, in combination with a Waters Acquity UPLC is used.

8.2 MS methods.
The MS methods have been optimised during validation for the Waters TQ-S system. The MS methods have been installed at the TQ-S system.

8.2.1 Tune method: SOPA1061.

Tune settings:

- Source temperature: 150ºC.
- Desolvation gas temperature: 600ºC.
- Desolvation gas flow: 800 L/h.
- Cone gas flow: 150 L/h.
- Cone voltage: 30 V.
- Capillary voltage: 3.0 kV.
- Argon collision gas pressure: 4.0 mbar.

8.2.2 Inlet method: TAs_EFSA_2015.

UPLC conditions:

- Column: Waters UPLC BEH C18 150 x 2.1 mm, 1.7 µm (Waters 186002353).
- Column temperature: 50ºC.
- Mobile phase A: 10 mM ammonium carbonate pH 10.0 (3.2.5).
- Mobile phase B: acetonitrile (3.2.6).
- Flow: 0.4 ml/min.
- Injection volume: 2 µl.
- Total run time: 15 min.
- Gradient (linear):

Time (min)	Mobile phase A (3.2.5)	Mobile phase B (3.2.6)
0.0	100%	0%
2.0	100%	0%
12.0	40%	60%
12.2	100%	0%
15.0	100%	0%

Note: injection of larger volumes of extract may result in strong matrix suppression effects and/or distorted peak shapes for the low molecular weight TAs.

8.2.3 MS method: TAs_EFSA_2015.

MS/MS fragmentation conditions used in MRM method:

Compound	$[M+H]^+$ *m/z*	Frag 1 *m/z*	CE eV	Frag 2 *m/z*	CE eV	Frag 3 *m/z*	CE eV	RT min	MRM
Nortropinone	126.1	67.0	20	68.0	15	**84.0**	**15**	4.40	1
Tropinone	140.1	57.0	25	82.0	15	**98.0**	**20**	5.45	1
Tropine	142.1	58.0	20	93.0	20	**98.0**	**20**	4.20	1
Pseudotropine	142.1	67.0	20	**96.0**	**20**	**124.0**	**15**	4.85	1
6-OH-tropinone	156.1	58.0	20	81.0	20	**98.0**	**15**	3.90	1
Scopine	156.1	73.0	15	**84.0**	**15**	98.0	15	4.05	1
Scopoline	156.1	81.0	20	110.0	20	**138.0**	**15**	4.95	1
Phenylacetoxytropane	260.2	91.0	35	93.0	25	**124.0**	**20**	10.45	2
Apoatropine	272.2	91.0	35	93.0	25	**124.0**	**20**	11.40	2
Aposcopolamine	286.2	103.0	20	110.0	20	**138.0**	**20**	10.35	2
Convolamine	306.2	91.0	35	93.0	25	**124.0**	**25**	9.70	2
Acetylscopolamine	346.2	103.0	20	**138.0**	**20**	286.0	20	9.85	2

Noratropine	276.2	91.0	30	**93.0**	**25**	110.0	20	7.70	3
Homatropine	276.2	93.0	25	**124.0**	**20**	142.0	25	8.45	3
Norscopolamine	290.2	**121.0**	**20**	124.0	20	**142.0**	**20**	7.25	3
Littorine	290.2	93.0	25	**124.0**	**20**	142.0	20	9.20	3
Atropine	290.2	91.0	35	93.0	25	**124.0**	**20**	8.70	3
Atropine-d3	293.2	93.0	25	**127.0**	**20**			8.65	3
Scopolamine	304.2	103.0	35	**138.0**	**20**	156.0	25	8.00	3
Scopolamine-d3	307.2	**141.0**	**20**	159.0	25			8.00	3
Convolidine	278.2	**93.0**	**20**	110.0	20	151.0	30	5.35	4
Fillalbine	292.2	91.0	35	93.0	25	**124.0**	**20**	6.35	4
Convolvine	292.2	**93.0**	**20**	110.0	20	165.0	30	8.65	4
Anisodamine	306.2	91.0	35	122.0	25	**140.0**	**20**	7.30	4
Anisodine	320.2	91.0	35	**138.0**	**20**	**156.0**	**20**	7.30	4
a-HO-methylatropine	320.2	91.0	35	93.0	35	**124.0**	**25**	7.75	4

In bold: most intense fragment

MRM windows:

- MRM1: 1.5-6.5 min.
- MRM2: 9.0-13.5 min.
- MRM3: 6.5-13.5 min.
- MRM4: 1.5-9.0 min.

8.4 Injection order of series.

- Working standard (2-3x).
- Mobile phase.
- MMS (8 samples).
- MMRS (sample injected in duplicate).
- Mobile phase.
- QC (2 samples).
- Mobile phase.
- Sample extracts.
- Mobile phase.
- MMS (8 samples).

Part D: Confirmation of positive samples and reporting

9. <u>Calculation, confirmation and reporting</u>

9.1 Calculation of concentration in the sample using MMS calibration curves

Calculate the concentration in the sample (ng/g) by comparing the sum of the peak area in the sample with the calibration curve prepared from the WS series, constructed by means of the least squares method for linear regression.

9.2 Calculation of concentration in the sample using standard addition to the sample (stir-fry vegetable products)

$$C\ \left({}^{\mu g}/_{kg}\right) = \frac{area_{sample}}{area_{sample+addition} - area_{sample}} \times C_{addition}({}^{\mu g}/_{kg})$$

9.3 Samples containing one or more TA above the LOQ

When in a sample atropine or scopolamine is found above the LOQ, the sample should be reanalysed by taking a new, unused sub-sample. The reported result is calculated as the average of the two measurements.
When in a sample a TA other than atropine or scopolamine is found above the LOQ, the sample should be reanalysed by means of standard addition to the sample, by taking a new, unused sub-sample. The amount of standard added to the sample should be between 2 and 5 times the estimated concentration present in the sample. The reported result is the amount calculated based on the standard addition.

Alternatively the sample can be sent to RIKILT for further confirmation. RIKILT will re-analyse those samples by standard addition.

10. Data analysis and storage

- Raw MS data are processed with Waters Quanlynx 4.1 software.
- Quanlynx data are transported automatically to the Report forms in excel format designed for the analysis of TAs in cereal (products).
- Quantification and acceptance of results is conducted according to criteria described in SANCO/12571/2013.
- Raw MS data are stored on the designated data server.

REPORT

Tropane Alkaloids in Cereals – EFSA Survey

Validation Report of RIKILT Method

FS 102116

July 2016

J. Stratton, J. Clough, M. Sehnalova, A. Lloyd, I. Leon, M. Parmar and S. MacDonald

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Annex 3. Fera Validation report for tropane alkaloids in cereal products

Summary

A method validation study for a method for tropane alkaloids in cereal products was completed. The method uses Strong Cation Exchange Solid Phase Extraction Clean-up (SCX SPE) followed by determination by UPLC-MS/MS.

The validation data showed variable performance of the method for different analytes. The two main analytes of concern that will be regulated (atropine and scopolamine) showed accepted method performance when compared to method performance criteria from Regulation (EU) No 401/2006. The better performance of these analytes can be attributed to the use of isotopically labelled internal standards for these two compounds.

The other analytes gave variable performance; some met the criteria from Regulation (EU) 401/2006, while others did not. The most common problem was occurrence of interferences in the chromatography for the early eluting compounds.

A modification to the initial clean-up procedure was made for this validation to reduce these interferences. This worked to some degree. The other change that helped improve the chromatography was the use of ammonium carbonate in the LC Mobile phase. It was found that the pH was critical to the chromatographic performance of the method, and that pH 10 and above gave the best results in terms of peak shape and separation.

In the previous validation it was shown that the use of matrix matched calibration series did not appear to assist with the analysis of different samples with many ingredients and complex matrices. Therefore solvent calibration standards were used for this validation and for the analysis of the survey samples. All survey samples will be overspiked to determine apparent recovery.

1 Introduction

This report describes the procedure and the performance characteristics of a test method for the LC-MS analysis of 24 tropane alkaloids in cereals and cereal products. The tropane alkaloids analysed using this method are 6-hydroxytropinone, nortropinone, pseudotropine, scopine, scopoline, tropine, tropinone, acetylscopolamine, anisodamine, anisodine, apoatropine, aposcopolamine, atropine, convolamine, convolidine, convolvine, fillalbin, homatropine, α-hydroxymethylatropine, littorine, noratropine, norscopolamine, phenylacetoxytropane and scopolamine. D_3-atropine and D_3-scopolamine are used as internal standards.

Validation of the method was performed by the analysis of six matrices, each with six replicate spikes at two concentrations (1 ng/g and 5 ng/g), quantified using solvent calibration standards. The matrices used for the validation were three breakfast cereals (multi-grain based cereal for young children, wheat bran flakes for breakfast and muesli with fruit and nuts), plain flours, bread and cookies. Each matrix was analysed alongside QC samples prepared by spiking a similar matrix at 10 ng/g and also alongside QC samples spiked by RIKILT. Recovery was assessed via the inclusion of extracts spiked after extraction and clean-up at a concentration equivalent to 10 ng/g.

Annex 3. Fera Validation report for tropane alkaloids in cereal products

The limit of detection (LOD) of the method was defined as the concentration of each analyte required to give a chromatographic peak with signal to noise (s:n) ratio ≥ 3. This was assessed by extrapolation from the s:n ratio of each analyte in the lowest matrix-extracted calibration standard. If there was a peak present in the blank (e.g. due to injection carry-over), the LOD was defined as the concentration required to give a peak area ≥ 2 x that in the blank. The limit of quantification (LOQ) of the method was defined as the concentration of the lowest calibration standard with s:n ≥ 3 and with a peak area fitting a calibration line with residuals ≤ 30 % and $r^2 \geq 0.95$. In cases where the calibration graph did not meet these criteria, the LOQ reported is the concentration of the lowest calibration standard included in the graph used to generate the indicative concentrations reported.

2 Materials and Methods

Sample extraction, clean-up and analysis were performed following *Standard Operation Protocol for LC-MS/MS analysis of TAs in cereals, cereal products, (herbal) dry tea, (herbal) tea infusions and stir-fry vegetable products, SOP for EFSA project Occurrence of TAs in food, Patrick Mulder & Patricia López Sánchez, February 2016, version g*. This SOP is attached as Annex 2. Any deviations from the SOP are recorded below. The validation protocol used by all partner laboratories is included here as Appendix I to this validation report.

2.1 Standards

Stock solutions of the individual tropane alkaloids at 100 µg/mL were provided by RIKILT. A mixed standard at 1000 ng/mL was prepared by pipetting 200 µL of each stock into a 20 mL volumetric flask and making up to the mark with methanol. This mixed standard was then further diluted to 100 ng/mL by pipetting 1 mL into a 10 mL volumetric flask and making up to the mark with methanol and to 10 ng/mL by pipetting 100 µL into a 10 mL volumetric flask and making up to the mark with methanol.

Stock solutions of the individual internal standards at 100 µg/mL were also provided by RIKILT. A mixed internal standard solution at 1000 ng/mL was prepared by pipetting 250 µL of each stock into a 25 mL volumetric flask and making up to the mark with methanol.

This method of preparing the mixed standard solutions differs from the SOP (see SOP sections 1.2-2.2).

2.2 Reagents

All reagents used were of an equivalent quality to that specified in the SOP (Annex 2 section 3) and mixed solvents were prepared in the same way (scaling up or down for quantity required). The elution solvent used was 1 % ammonia in methanol, which was prepared by dilution of 7 N ammonia in methanol (21 mL 7 N ammonia in methanol was mixed with 229 mL methanol). This method of preparation differs from the SOP (Annex 2 section 3.2.4). The mobile phases used were those specified for the "alternative UPLC conditions" in the SOP (Annex 2 7.2.2), namely (A) 10 mM ammonium carbonate and (B) acetonitrile.

2.3 Equipment

The equipment used was equivalent to that specified in the SOP (Annex 2 section 4) with the following exceptions:

An orbital shaker (AQS) was used instead of an overhead shaker.

Samples were dried under nitrogen on a dry block sample concentrator, not in a TurboVap.

Samples were collected after elution and dried down in glass sample tubes instead of polypropylene.

Samples were filtered using 0.2 µm nylon syringe filters into glass vials instead of using 0.45 µm polypropylene PTFE filtervials. A smaller particle filter was required to use with the UPLC column and it was found that the samples would not pass through 0.2 µm PTFE filters, possibly due to swelling caused by the aqueous solvent.

The SPE cartridges chosen were Phenomenex Strata-X-C, 33 µm, 200 mg, 6 mL.

2.4 Sample Extraction and Clean-Up

Sample extraction and clean-up was performed following the SOP (Annex 2 section 6), with the changes to equipment noted above. An additional change from the SOP was that buckwheat extracts were microcentrifuged at 12,000 rpm for 5 minutes after reconstitution to avoid blocking the 0.22 µm nylon syringe filters.

The calibration standards concentrations, samples and spikes included in each batch followed *Protocol for the validation of LC-MS/MS analysis of TAs in breakfast cereals, plain flours, bread and cookies, Patrick Mulder, Version g* (see Annex 2). The QC included with each validation batch therefore differs slightly from that specified in the SOP. The matrices used are shown in Table 1. Calibration standards were prepared in solvent in a range equivalent to 0.1-50 ng/g (0.1-50 ng/mL in the vial). The use of calibration standards prepared in solvent deviates from the SOP, which specifies the use of matrix extracted standards.

Table 1: Matrices used for validation spikes and QC in validation batches.

Validation Matrix	QC1	QC2	Rikilt QC
Infant multigrain cereal	Toasted wheat branflakes	Fruit & nut muesli	Cereal
Toasted wheat branflakes	Infant multigrain cereal	Fruit & nut muesli	Cereal
Fruit & nut muesli	Infant multigrain cereal	Toasted wheat branflakes	Cereal
Buckwheat	Millet flakes	Sorghum	Buckwheat
Wholemeal rolls	Seed sensations granary bread	White baguette	Bread
Biscuits with fruit	Rich tea	Infant chocolate biscotti	Cookie

Validation batches for each matrix were prepared and run separately. For each batch, weighing, spiking and extraction were performed on one day and the extracts collected after centrifugation and stored in the fridge overnight. After SPE clean-up, evaporation and reconstitution the sample vials were stored in the fridge for a maximum of two nights before LC-MS analysis. They were not stored in the freezer as specified in the SOP (Annex 2 section 6.4) due to concerns about expansion of the aqueous sample in the insert vial on freezing.

Matrix-matched recovery samples (MMRS) were prepared by taking two additional aliquots of the validation matrix through the clean-up and evaporation procedure and then adding 10 µL of the tropane alkaloid mixed standard at 1000 ng/mL, 40 µL methanol and 450 µL water. This method differs from the SOP (Annex 2 section 6.4).

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2.5 LC-MS Analysis

Samples were analysed using an Acquity UPLC system coupled to a Xevo TQ-S tandem quadrupole mass spectrometer (Waters).

2.5.1 LC Method

Column: Acquity BEH C_{18} 1.7 µm (150 x 2.1 mm) (Waters)

Column temperature: 50 °C

Injection volume: 2 µL

Mobile phase A: 10 mM ammonium carbonate in water

Mobile phase B: Acetonitrile

Gradient timetable:

Time / min	% B
0.0	0
2.0	0
12.0	50
12.2	100
16.0	100
16.2	0
20.0	0

The gradient profile differs from that in the SOP (SOP section 7.2.2) by the addition of the flush step at 100 % B.

2.5.2 MS Method

Ionisation mode: Electrospray positive

Capillary voltage: 1 kV

Desolvation temperature: 500 °C

Desolvation gas flow rate: 1000 L/h

Nebuliser gas flow: 7 bar

Source temperature: 150 °C

Cone gas flow rate: 100 L/h

Collision gas flow rate: 0.15 mL/min (3.5 mbar)

Resolution: Unit mass

MRM conditions: As SOP (section 7.2.3)

These source tune parameters differ slightly from those in the SOP (Annex 2 section 7.2.1)

3 Results and Discussion

Chromatographic peak shapes were generally improved compared to the previous validation. However, some of the issues noted during the initial validation were still observed. For example, tailing was observed for some analytes (particularly tropine, scopoline, convolidine and fillalbin). Many of the problems were associated with the low molecular weight, early-eluting compounds as these elute with very little or no organic solvent in the mobile phase and would probably benefit from being run under completely different chromatography conditions. Changes in peak shapes were also observed when comparing matrix and solvent, or during a batch. Because it was known that all the samples contained the analytes it was possible, for these batches, to work out which peak should be integrated, although consistent integration was often very difficult. Identifying whether a peak is the correct one will be much more difficult for survey samples.

Despite the modifications to the clean-up procedure to improve washing and elution, there were numerous interferences in some chromatograms, and in several cases these interferences were not fully resolved from the analyte peaks which made consistent integration of the peaks difficult. This was particularly true for tropine and nortropinone. In several cases an interference made it necessary to raise the LOD and/or LOQ for that analyte to a concentration where the contribution from this interference would be minimal. Interferences had a much larger influence on the LOD and LOQ than the actual response of the instrument. The instrument sensitivity for these analytes is very good, with low (sub ng/g) concentrations giving good signal to noise. Therefore it may be possible to reduce the interferences and improve the method performance further by reducing the amount of sample extract loaded on the SPE cartridges (to prevent overloading the cartridges with matrix) or by making a further dilution of the cleaned up extract after re-constitution to dilute matrix effects. Dilution of extracts was assessed for some samples and it did improve the chromatography observed.

Carry-over into the next injection(s) was observed for some analytes. Matrix washes were used to minimise carry-over into the samples as this seemed more effective than a simple solvent wash. In many cases the problem of carry-over was compounded by negative intercepts on the calibration graph which gave an unrealistically high calculated concentration for relatively small carry-over peaks.

Results for the analysis of each analyte in the six matrices included in the validation are shown in tables 2 to 7.

The recovery values reported for the QC samples are corrected using the associated blank except where any residue in the blank appears to be caused by carry-over from a previous injection (i.e. where there is a similar residue in the preceding wash).

Because the validation spikes were quantified using solvent calibration standards the value reported as ‘recovery’ is actually a combination of recovery and the relative response of the analyte in matrix compared with solvent (i.e. matrix suppression or enhancement of signal). The RIKLT QC samples give a separate indication of recovery as these were pre-spiked materials received from RIKILT, but again these results give combined recovery and relative response of analyte in matrix compared with solvent. The actual recovery of the extraction procedure can be measured by comparison of the MMRS samples, which were spiked after extraction and clean-up, with samples spiked before extraction and clean-up. The MMRS samples were spiked at a level of 10ng/g.

These values are, for the most part, within an acceptable range, although tropinone and acetylscopolamine recovery was below 50% for several products. Buckwheat flour was a problematic matrix with very low apparent recovery for many analytes, although true recovery from MMRS spikes was acceptable. This highlights the problems of matrix suppression on analyte signal : noise and also explains the variation in LOD/LOQ and variation in spikes observed between many of the products. These are caused by interferences and difficulties with peak shapes, not by poor recovery. Buckwheat is one matrix that would benefit from using a reduced amount of sample extract for clean-up as the matrix is so complex it seems it may have overloaded the clean-up cartridges as the final extracts were not as well cleaned-up as other matrices and were often difficult to reconstitute and filter.

4 Conclusions

The method gave acceptable performance for atropine and scopolamine. In all cases the performance criteria for recovery and repeatability from Regulation (EU) No 401/2006 were met except for muesli and bread where the average recovery values exceeded 120%, with highest level of 131% for the 1 ng/g scopolamine spikes in bread which is outside the 60 to 120% recommended range. In all cases the MMRS recovery was acceptable.

Limits of detection (LOD) and quantification (LOQ) were also acceptable for atropine and scopolamine.

For the other analytes in many cases the method performance for the MMRS recovery was acceptable according to the criteria in Regulation (EU) No 401/2006, however the recovery for tropinone was consistently around 50% or less. The method is very repeatable for all analytes, RSD values for replicate spikes were consistently below 10%, the maximum variability observed was 13% which is well within the acceptable performance criteria.

It was recommended to use a matrix extracted calibration series for the initial validation and survey. The original validation data showed that unless the calibration matrix is exactly the same as the test samples this has no benefit and in fact can make results appear worse. For this validation and the analysis of the survey samples solvent calibration standards have been used. For the survey samples an overspike of each test sample was also prepared to allow a positive control of every sample and to gain information on individual recovery values.

In its current form the method is suitable for atropine and scopolamine but the results for some of the other analytes may still have a degree of uncertainty due to interferences observed in chromatography. This is not so apparent during validation as all samples are spiked, but becomes more of an issue for peak identification for test samples.

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Table 2. Validation results for tropane alkaloids in Multigrain cereal (low molecular weight)

Sample Type	Description	Nortropinone	Tropinone	Tropine	Pseudotropine	6-hydroxytropinone	Scopine	Scopoline
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Multi-grain cereal for children	Blank	0.06	0.00	0.00	0.04	0.00	0.00	0.00
Multi-grain cereal for children	Spike 1 1ng/g	0.62	0.31	0.74	0.87	0.69	0.72	0.54
Multi-grain cereal for children	Spike 2 1ng/g	0.61	0.32	0.74	0.79	0.68	0.83	0.57
Multi-grain cereal for children	Spike 3 1ng/g	0.61	0.33	0.78	0.87	0.68	0.80	0.61
Multi-grain cereal for children	Spike 4 1ng/g	0.60	0.33	0.77	0.84	0.67	0.74	0.59
Multi-grain cereal for children	Spike 5 1ng/g	0.63	0.33	0.83	0.86	0.65	0.79	0.60
	Mean	**0.55**	**0.33**	**0.77**	**0.81**	**0.68**	**0.78**	**0.58**
	"Recovery"	**55%**	**33%**	**77%**	**81%**	**68%**	**78%**	**58%**
	RSD	**2%**	**3%**	**4%**	**4%**	**2%**	**6%**	**5%**
Multi-grain cereal for children	Spike 6 5ng/g	2.67	1.92	4.14	3.98	3.33	4.21	3.32
Multi-grain cereal for children	Spike 7 5ng/g	2.90	1.64	4.26	4.07	3.57	4.34	3.46
Multi-grain cereal for children	Spike 8 5ng/g	2.74	1.79	4.29	4.27	3.61	4.61	3.38
Multi-grain cereal for children	Spike 9 5ng/g	2.68	1.72	4.21	3.92	3.34	4.17	3.37
Multi-grain cereal for children	Spike 10 5ng/g	2.98	1.83	4.30	4.11	3.50	4.40	3.45
	Mean	**2.73**	**1.78**	**4.24**	**4.03**	**3.47**	**4.35**	**3.40**
	"Recovery"	**55%**	**36%**	**85%**	**81%**	**69%**	**87%**	**68%**
	RSD	**5%**	**6%**	**2%**	**3%**	**4%**	**4%**	**2%**
Multi-grain cereal for children	MMRS 1 10 ng/g	7.10	9.27	8.17	8.10	8.12	7.47	8.47
Multi-grain cereal for children	MMRS 2 10 ng/g	7.96	10.55	10.23	9.36	8.94	9.27	9.73
	Mean	**7.47**	**9.91**	**9.20**	**8.69**	**8.53**	**8.37**	**9.10**
	Recovery	**73%**	**36%**	**92%**	**93%**	**81%**	**104%**	**75%**
Cereal	Rikilt QC Blank	0.16	0.02	0.00	0.04	0.00	0.00	0.00
Cereal	Rikilt QC Spike 10 ng/g	3.10	18.35	5.88	4.81	6.86	4.90	8.61
	"Recovery"	**15%**	**92%**	**29%**	**24%**	**34%**	**25%**	**43%**
Wheat bran	QC1 Blank	0.00	0.00	0.00	0.08	0.00	0.00	0.00
Wheat bran	QC1 Spike 10 ng/g	3.63	2.53	7.49	7.17	8.46	7.89	4.51
	"Recovery"	**36%**	**25%**	**75%**	**71%**	**85%**	**79%**	**45%**
Muesli with fruit and nuts	QC2 Blank	0.13	0.00	0.00	0.05	0.00	0.00	0.00
Muesli with fruit and nuts	QC2 Spike 10 ng/g	1.94	4.46	3.53	4.80	3.46	3.62	4.49
	"Recovery"	**18%**	**45%**	**35%**	**47%**	**35%**	**36%**	**45%**

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Table 2 (continued): Validation results for tropane alkaloid in multigrain cereal (convolamine type).

Sample Type	Description	3a-Phenylacetoxytropane	Convolidine	Fillalbin	Convolvine	Convolamine
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Multi-grain cereal for children	Blank	0.01	0.00	0.00	0.00	0.05
Multi-grain cereal for children	Spike 1 1ng/g	0.81	(0.91)	(0.99)	0.56	0.96
Multi-grain cereal for children	Spike 2 1ng/g	0.65	(0.84)	(1.00)	0.56	0.86
Multi-grain cereal for children	Spike 3 1ng/g	0.66	(0.87)	(1.06)	0.57	0.88
Multi-grain cereal for children	Spike 4 1ng/g	0.64	(0.85)	(1.00)	0.56	0.87
Multi-grain cereal for children	Spike 5 1ng/g	0.70	(0.91)	(1.04)	0.60	0.93
	Mean	**0.68**	**0.88**	**1.02**	**0.57**	**0.90**
	"Recovery"	**68%**	**88%**	**102%**	**57%**	**90%**
	RSD	**10%**	**4%**	**3%**	**4%**	**5%**
Multi-grain cereal for children	Spike 6 5ng/g	3.31	(3.75)	(4.47)	2.79	4.28
Multi-grain cereal for children	Spike 7 5ng/g	3.32	(3.93)	(4.56)	2.82	4.33
Multi-grain cereal for children	Spike 8 5ng/g	3.36	(3.93)	(4.62)	2.86	4.52
Multi-grain cereal for children	Spike 9 5ng/g	3.33	(3.86)	(4.46)	2.82	4.40
Multi-grain cereal for children	Spike 10 5ng/g	3.27	(3.93)	(4.61)	2.83	4.37
	Mean	**3.31**	**3.88**	**4.55**	**2.82**	**4.38**
	"Recovery"	**66%**	**78%**	**91%**	**56%**	**88%**
	RSD	**1%**	**2%**	**2%**	**1%**	**2%**
Multi-grain cereal for children	MMRS 1 10 ng/g	9.11	(9.43)	(9.57)	6.72	(10.35)
Multi-grain cereal for children	MMRS 2 10 ng/g	7.59	(9.19)	(10.59)	7.61	(9.75)
	Mean	**8.34**	**9.31**	**10.08**	**7.16**	**10.05**
	Recovery	**79%**	**83%**	**90%**	**79%**	**87%**
Cereal	Rikilt QC Blank	0.00	0.00	0.00	0.00	0.00
Cereal	Rikilt QC Spike 10 ng/g	8.29	(5.90)	(9.16)	7.43	(13.75)
	"Recovery"	**83%**	**29%**	**46%**	**37%**	**69%**
Wheat bran	QC1 Blank	0.01	0.00	0.00	0.00	0.05
Wheat bran	QC1 Spike 10 ng/g	7.43	(6.48)	(8.38)	4.89	8.25
	"Recovery"	**74%**	**65%**	**84%**	**49%**	**83%**
Muesli with fruit and nuts	QC2 Blank	0.01	0.00	0.00	0.00	0.00
Muesli with fruit and nuts	QC2 Spike 10 ng/g	8.58	(4.25)	(8.65)	6.58	9.61
	"Recovery"	**86%**	**42%**	**87%**	**66%**	**96%**

Table 2 (continued): Validation results for tropane alkaloids in Multigrain cereal (atropine type part 1).

Sample Type	Description	Apoatropine	Noratropine	Homatropine	Aposcopolamine	Norscopolamine	Atropine	Atropine D3
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Rel Resp
Multi-grain cereal for children	Blank	0.05	0.01	0.00	0.00	0.00	0.00	48%
Multi-grain cereal for children	Spike 1 1ng/g	0.86	0.57	0.44	0.70	0.82	1.20	47%
Multi-grain cereal for children	Spike 2 1ng/g	0.71	0.59	0.44	0.56	0.81	1.17	48%
Multi-grain cereal for children	Spike 3 1ng/g	0.72	0.59	0.46	0.57	0.81	1.21	48%
Multi-grain cereal for children	Spike 4 1ng/g	0.70	0.58	0.46	0.55	0.80	1.14	50%
Multi-grain cereal for children	Spike 5 1ng/g	0.77	0.60	0.48	0.60	0.81	1.16	51%
	Mean	**0.75**	**0.59**	**0.46**	**0.59**	**0.81**	**1.18**	**49%**
	"Recovery"	**75%**	**59%**	**46%**	**59%**	**81%**	**118%**	**49%**
	RSD	**9%**	**2%**	**4%**	**10%**	**1%**	**3%**	**4%**
Multi-grain cereal for children	Spike 6 5ng/g	3.56	2.96	2.19	2.97	4.12	6.01	48%
Multi-grain cereal for children	Spike 7 5ng/g	3.59	3.00	2.22	3.04	4.11	5.89	51%
Multi-grain cereal for children	Spike 8 5ng/g	3.53	3.08	2.26	2.88	4.11	5.83	51%
Multi-grain cereal for children	Spike 9 5ng/g	3.52	3.00	2.25	2.94	4.11	5.98	50%
Multi-grain cereal for children	Spike 10 5ng/g	3.47	2.98	2.27	2.94	4.20	5.92	51%
	Mean	**3.54**	**3.00**	**2.24**	**2.95**	**4.13**	**5.93**	**50%**
	"Recovery"	**71%**	**60%**	**45%**	**59%**	**83%**	**119%**	**50%**
	RSD	**1%**	**1%**	**1%**	**2%**	**1%**	**1%**	**2%**
Multi-grain cereal for children	MMRS 1 10 ng/g	(11.80)	6.10	4.43	8.14	7.77	(12.13)	48%
Multi-grain cereal for children	MMRS 2 10 ng/g	9.60	6.90	4.99	6.83	8.56	(13.31)	49%
	Mean	**10.70**	**6.50**	**4.71**	**7.48**	**8.17**	**12.72**	**48%**
	Recovery	**66%**	**92%**	**95%**	**79%**	**101%**	**93%**	**105%**
Cereal	Rikilt QC Blank	0.00	0.00	0.00	0.00	0.00	0.16	37%
Cereal	Rikilt QC Spike 10 ng/g	(14.07)	5.52	5.74	9.35	5.18	(22.77)	38%
	"Recovery"	**70%**	**28%**	**29%**	**47%**	**26%**	**113%**	**38%**
Wheat bran	QC1 Blank	0.00	0.01	0.00	0.00	0.00	0.00	42%
Wheat bran	QC1 Spike 10 ng/g	7.32	5.32	4.22	5.71	6.31	(12.37)	45%
	"Recovery"	**73%**	**53%**	**42%**	**57%**	**63%**	**124%**	**45%**
Muesli with fruit and nuts	QC2 Blank	0.00	0.01	0.00	0.00	0.00	0.00	56%
Muesli with fruit and nuts	QC2 Spike 10 ng/g	8.28	5.43	5.20	5.84	6.17	(12.32)	56%
	"Recovery"	**83%**	**54%**	**52%**	**58%**	**62%**	**123%**	**56%**

Table 2 (continued): Validation results for tropane alkaloids in Multigrain cereal (atropine type part 2).

Sample Type	Description	Littorine	Scopolamine	Scopolamine D3	Anisodamine	Anisodine	2a-OH-methylatropine	o-Acetylscopolamine
		Conc / ng/g	Conc / ng/g	Rel Resp	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Multi-grain cereal for children	Blank	0.00	0.00	45%	0.00	0.00	0.00	0.00
Multi-grain cereal for children	Spike 1 1ng/g	0.63	1.13	45%	0.88	0.89	0.65	0.62
Multi-grain cereal for children	Spike 2 1ng/g	0.61	1.12	47%	0.91	0.92	0.68	0.52
Multi-grain cereal for children	Spike 3 1ng/g	0.62	1.15	45%	0.91	0.91	0.69	0.56
Multi-grain cereal for children	Spike 4 1ng/g	0.62	1.07	48%	0.90	0.92	0.69	0.50
Multi-grain cereal for children	Spike 5 1ng/g	0.67	1.14	47%	0.94	0.93	0.72	0.56
	Mean	**0.63**	**1.12**	**47%**	**0.91**	**0.91**	**0.68**	**0.55**
	"Recovery"	**63%**	**112%**	**47%**	**91%**	**91%**	**68%**	**55%**
	RSD	**4%**	**3%**	**3%**	**2%**	**2%**	**4%**	**8%**
Multi-grain cereal for children	Spike 6 5ng/g	3.07	6.09	45%	4.57	4.65	3.44	2.79
Multi-grain cereal for children	Spike 7 5ng/g	3.11	5.73	48%	4.64	4.70	3.54	2.80
Multi-grain cereal for children	Spike 8 5ng/g	3.20	5.78	51%	4.72	4.80	3.66	2.66
Multi-grain cereal for children	Spike 9 5ng/g	3.16	5.85	48%	4.66	4.80	3.58	2.94
Multi-grain cereal for children	Spike 10 5ng/g	3.19	5.71	49%	4.71	4.92	3.61	2.94
	Mean	**3.15**	**5.83**	**48%**	**4.66**	**4.77**	**3.56**	**2.83**
	"Recovery"	**63%**	**117%**	**48%**	**93%**	**95%**	**71%**	**57%**
	RSD	**2%**	**3%**	**4%**	**1%**	**2%**	**2%**	**4%**
Multi-grain cereal for children	MMRS 1 10 ng/g	6.34	9.73	46%	8.79	8.70	6.64	9.37
Multi-grain cereal for children	MMRS 2 10 ng/g	6.73	(10.83)	47%	9.85	9.77	7.44	8.68
	Mean	**6.53**	**10.28**	**47%**	**9.32**	**9.24**	**7.03**	**9.03**
	Recovery	**96%**	**113%**	**103%**	**100%**	**103%**	**101%**	**63%**
Cereal	Rikilt QC Blank	0.00	0.27	31%	0.00	0.00	0.00	0.00
Cereal	Rikilt QC Spike 10 ng/g	9.70	(18.74)	32%	6.68 ^	7.04	3.35	(11.64)
	"Recovery"	**49%**	**92%**	**32%**	**33%**	**35%**	**34%**	**58%**
Wheat bran	QC1 Blank	0.00	0.00	41%	0.00	0.00	0.00	0.00
Wheat bran	QC1 Spike 10 ng/g	5.67	(10.66)	41%	7.21	7.40	6.47	7.23
	"Recovery"	**57%**	**107%**	**41%**	**72%**	**74%**	**65%**	**72%**
Muesli with fruit and nuts	QC2 Blank	0.00	0.14	55%	0.00	0.00	0.00	0.00
Muesli with fruit and nuts	QC2 Spike 10 ng/g	6.84	(10.58)	53%	7.23	8.01	6.85	7.98
	"Recovery"	**68%**	**104%**	**53%**	**72%**	**80%**	**69%**	**80%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 3. Validation results for tropane alkaloids in Wheatbran (low molecular weight))

Sample Type	Description	Nortropinone	Tropinone	Tropine	Pseudotropine	6-hydroxytropinone	Scopine	Scopoline
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Wheat bran	Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Wheat bran	Spike 1 1ng/g	0.34	0.30	0.70 ^	0.76	0.49	0.59	0.51
Wheat bran	Spike 2 1ng/g	0.35	0.30	0.73 ^	0.77	0.51	0.61	0.52
Wheat bran	Spike 3 1ng/g	0.35	0.29	0.71 ^	0.80	0.49	0.60	0.53
Wheat bran	Spike 4 1ng/g	0.38	0.32	0.72 ^	0.76	0.53	0.62	0.55
Wheat bran	Spike 5 1ng/g	0.46	0.36	0.70 ^	0.80	0.55	0.62	0.60
	Mean	**0.38**	**0.31**	**0.71**	**0.78**	**0.51**	**0.61**	**0.54**
	"Recovery"	**38%**	**31%**	**71%**	**78%**	**51%**	**61%**	**54%**
	RSD	**13%**	**8%**	**2%**	**2%**	**5%**	**2%**	**6%**
Wheat bran	Spike 6 5ng/g	2.56	2.21	3.64	4.07	3.08	3.26	3.05
Wheat bran	Spike 7 5ng/g	2.57	2.22	3.70	4.23	3.02	3.35	3.06
Wheat bran	Spike 8 5ng/g	2.26	1.56	3.41	3.97	2.80	3.05	2.70
Wheat bran	Spike 9 5ng/g	2.66	1.91	3.52	4.16	3.16	3.23	3.11
Wheat bran	Spike 10 5ng/g	2.58	1.72	3.41	4.00	3.08	3.35	3.06
	Mean	**2.53**	**1.92**	**3.53**	**4.09**	**3.03**	**3.25**	**3.00**
	"Recovery"	**51%**	**38%**	**71%**	**82%**	**61%**	**65%**	**60%**
	RSD	**6%**	**15%**	**4%**	**3%**	**5%**	**4%**	**6%**
Wheat bran	MMRS 1 10 ng/g	6.25	7.98	7.77	8.86	7.33	7.05	7.36
Wheat bran	MMRS 2 10 ng/g	6.10	7.77	7.36	8.36	6.81	6.73	7.19
	Mean	**6.18**	**7.88**	**7.57**	**8.61**	**7.07**	**6.89**	**7.27**
	Recovery	**82%**	**49%**	**93%**	**95%**	**86%**	**94%**	**82%**
Cereal	Rikilt QC Blank	0.14	0.00	0.00	0.00	0.00	0.00	0.00
Cereal	Rikilt QC Spike 10 ng/g	2.63	16.16	8.08	9.11	4.80	2.16	7.62
	"Recovery"	**25%**	**162%**	**81%**	**91%**	**48%**	**22%**	**76%**
Multi-grain cereal for children	QC1 Blank	(0.05) ^	0.00	0.00	0.00	0.00	0.00	0.00
Multi-grain cereal for children	QC1 Spike 10 ng/g	4.68	4.00	7.36	9.58	4.11	6.43	6.99
	"Recovery"	**46%**	**40%**	**74%**	**96%**	**41%**	**64%**	**70%**
Muesli with fruit and nuts	QC2 Blank	0.13 ^	0.00	0.00	0.00	0.00	0.00	0.00
Muesli with fruit and nuts	QC2 Spike 10 ng/g	1.90	3.71	4.76	5.29	2.48	1.65	3.38
	"Recovery"	**18%**	**37%**	**48%**	**53%**	**25%**	**16%**	**34%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 3 (continued) Validation results for tropane alkaloids in Wheatbran (convolamine type)

Sample Type	Description	3a-Phenylacetoxytropane	Convolidine	Fillalbin	Convolvine	Convolamine
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Wheat bran	Blank	0.00	0.00	0.00	0.00	0.00
Wheat bran	Spike 1 1ng/g	1.24	0.51	0.71	1.03	1.36
Wheat bran	Spike 2 1ng/g	1.12	0.53	0.72	0.84	1.18
Wheat bran	Spike 3 1ng/g	1.11	0.52	0.73	0.81	1.19
Wheat bran	Spike 4 1ng/g	1.14	0.53	0.75	0.82	1.21
Wheat bran	Spike 5 1ng/g	1.13	0.53	0.74	0.82	1.20
	Mean	**1.15**	**0.52**	**0.73**	**0.86**	**1.23**
	"Recovery"	**115%**	**52%**	**73%**	**86%**	**123%**
	RSD	**5%**	**1%**	**2%**	**11%**	**6%**
Wheat bran	Spike 6 5ng/g	5.37	2.49	3.60	4.15	5.65
Wheat bran	Spike 7 5ng/g	5.82	2.58	3.66	4.27	6.12
Wheat bran	Spike 8 5ng/g	5.02	2.46	3.62	3.80	5.29
Wheat bran	Spike 9 5ng/g	5.42	2.54	3.53	4.07	5.75
Wheat bran	Spike 10 5ng/g	5.49	2.58	3.63	4.10	5.77
	Mean	**5.42**	**2.53**	**3.61**	**4.08**	**5.72**
	"Recovery"	**108%**	**51%**	**72%**	**82%**	**114%**
	RSD	**5%**	**2%**	**1%**	**4%**	**5%**
Wheat bran	MMRS 1 10 ng/g	(12.05)	5.44	7.89	9.38	(12.79)
Wheat bran	MMRS 2 10 ng/g	(12.13)	5.39	7.63	9.36	(12.50)
	Mean	**12.09**	**5.41**	**7.76**	**9.37**	**12.64**
	Recovery	**90%**	**94%**	**93%**	**87%**	**90%**
Cereal	Rikilt QC Blank	0.00	0.00	0.00	0.00	0.00
Cereal	Rikilt QC Spike 10 ng/g	9.51	5.16	9.33	(10.53)	(15.29)
	"Recovery"	**95%**	**52%**	**93%**	**105%**	**153%**
Multi-grain cereal for children	QC1 Blank	0.00	0.00	0.00	0.00	0.00
Multi-grain cereal for children	QC1 Spike 10 ng/g	(11.40)	6.64	8.64	(10.13)	(12.49)
	"Recovery"	**114%**	**66%**	**86%**	**101%**	**125%**
Muesli with fruit and nuts	QC2 Blank	0.00	0.00	0.00	0.00	0.00
Muesli with fruit and nuts	QC2 Spike 10 ng/g	(10.69)	2.84	7.26	8.77	(11.58)
	"Recovery"	**107%**	**28%**	**73%**	**88%**	**116%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 3 (continued): Validation results for tropane alkaloids in Wheatbran (atropine type part 1)

Sample Type	Description	Apoatropine	Noratropine	Homatropine	Aposcopolamine	Norscopolamine	Atropine	Atropine D3
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Rel Resp
Wheat bran	Blank	0.00	0.00	0.00	0.00	0.00	0.00	55%
Wheat bran	Spike 1 1ng/g	1.10	0.29	0.51	0.89	0.42	1.16	57%
Wheat bran	Spike 2 1ng/g	1.17	0.27	0.48	0.91	0.42	1.02	62%
Wheat bran	Spike 3 1ng/g	1.09	0.26	0.49	0.90	0.42	1.10	57%
Wheat bran	Spike 4 1ng/g	1.18	0.28	0.49	0.97	0.40	1.04	61%
Wheat bran	Spike 5 1ng/g	1.15	0.27	0.50	1.04	0.42	1.04	62%
	Mean	**1.14**	**0.27**	**0.49**	**0.94**	**0.42**	**1.07**	**60%**
	"Recovery"	**114%**	**27%**	**49%**	**94%**	**42%**	**107%**	**60%**
	RSD	**4%**	**5%**	**2%**	**7%**	**2%**	**5%**	**4%**
Wheat bran	Spike 6 5ng/g	5.70	1.43	2.41	5.27	2.20	5.23	61%
Wheat bran	Spike 7 5ng/g	5.85	1.44	2.54	5.39	2.23	5.31	63%
Wheat bran	Spike 8 5ng/g	4.84	1.41	2.31	4.43	2.28	5.08	59%
Wheat bran	Spike 9 5ng/g	5.73	1.40	2.36	5.12	2.19	5.51	57%
Wheat bran	Spike 10 5ng/g	5.67	1.29	2.36	4.96	2.21	5.28	61%
	Mean	**5.56**	**1.39**	**2.40**	**5.03**	**2.22**	**5.28**	**60%**
	"Recovery"	**111%**	**28%**	**48%**	**101%**	**44%**	**106%**	**60%**
	RSD	**7%**	**4%**	**4%**	**7%**	**2%**	**3%**	**3%**
Wheat bran	MMRS 1 10 ng/g	(16.90)	2.73	5.25	(10.66)	4.46	(13.63)	50%
Wheat bran	MMRS 2 10 ng/g	(14.51)	2.96	5.02	(10.39)	4.24	(12.45)	53%
	Mean	**15.71**	**2.84**	**5.13**	**10.53**	**4.35**	**13.04**	**52%**
	Recovery	**71%**	**98%**	**93%**	**96%**	**102%**	**81%**	**117%**
Cereal	Rikilt QC Blank	0.00	0.00	0.00	0.00	0.00	0.14	43%
Cereal	Rikilt QC Spike 10 ng/g	(20.52)	3.88	7.12	(12.80)	5.53	(20.86)	42%
	"Recovery"	**205%**	**39%**	**71%**	**128%**	**55%**	**207%**	**43%**
Multi-grain cereal for children	QC1 Blank	0.00	0.00	0.00	0.00	0.00	0.00	67%
Multi-grain cereal for children	QC1 Spike 10 ng/g	(11.39)	3.39	5.69	8.45	6.68	(10.19)	73%
	"Recovery"	**114%**	**34%**	**57%**	**85%**	**67%**	**102%**	**67%**
Muesli with fruit and nuts	QC2 Blank	0.00	0.00	0.00	0.00	0.00	0.00	68%
Muesli with fruit and nuts	QC2 Spike 10 ng/g	7.24	2.88	5.94	9.30	5.06	9.62	78%
	"Recovery"	**72%**	**29%**	**59%**	**93%**	**51%**	**96%**	**68%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 3 (continued): Validation results for tropane alkaloids in Wheatbran (atropine type part 2)

Sample Type	Description	Littorine	Scopolamine	Scopolamine D3	Anisodamine	Anisodine	2a-OH-methylatropine	o-Acetylscopolamine
		Conc / ng/g	Conc / ng/g	Rel Resp	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Wheat bran	Blank	0.00	0.00	36%	0.00	0.00	0.00	0.00
Wheat bran	Spike 1 1ng/g	0.80	1.29	37%	0.55	0.53	0.49	0.48
Wheat bran	Spike 2 1ng/g	0.79	1.17	41%	0.55	0.52	0.47	0.48
Wheat bran	Spike 3 1ng/g	0.79	1.23	38%	0.55	0.54	0.48	0.48
Wheat bran	Spike 4 1ng/g	0.78	1.12	42%	0.55	0.52	0.49	0.47
Wheat bran	Spike 5 1ng/g	0.79	1.15	42%	0.55	0.52	0.48	0.48
	Mean	**0.79**	**1.19**	**40%**	**0.55**	**0.53**	**0.48**	**0.48**
	"Recovery"	**79%**	**119%**	**40%**	**55%**	**53%**	**48%**	**48%**
	RSD	**1%**	**6%**	**5%**	**0%**	**1%**	**2%**	**1%**
Wheat bran	Spike 6 5ng/g	3.87	5.83	41%	2.81	2.76	2.47	2.57
Wheat bran	Spike 7 5ng/g	4.06	5.99	42%	2.85	2.77	2.50	2.68
Wheat bran	Spike 8 5ng/g	3.63	5.59	45%	2.85	2.81	2.50	1.97
Wheat bran	Spike 9 5ng/g	3.82	6.21	41%	2.78	2.74	2.33	2.35
Wheat bran	Spike 10 5ng/g	3.87	5.84	43%	2.80	2.69	2.34	2.40
	Mean	**3.85**	**5.89**	**42%**	**2.82**	**2.75**	**2.43**	**2.39**
	"Recovery"	**77%**	**118%**	**42%**	**56%**	**55%**	**49%**	**48%**
	RSD	**4%**	**4%**	**4%**	**1%**	**2%**	**4%**	**11%**
Wheat bran	MMRS 1 10 ng/g	8.33	(12.92)	33%	5.76	5.51	4.77	(11.01)
Wheat bran	MMRS 2 10 ng/g	8.30	(11.47)	36%	5.56	5.29	4.58	(10.81)
	Mean	**8.32**	**12.20**	**35%**	**5.66**	**5.40**	**4.68**	**10.91**
	Recovery	**93%**	**97%**	**122%**	**100%**	**102%**	**104%**	**44%**
Cereal	Rikilt QC Blank	0.00	0.33	22%	0.00	0.00	0.00	0.00
Cereal	Rikilt QC Spike 10 ng/g	8.25	(23.33)	24%	6.78	6.06	2.96	8.16
	"Recovery"	**83%**	**230%**	**22%**	**68%**	**61%**	**30%**	**82%**
Multi-grain cereal for children	QC1 Blank	0.00	0.00	45%	0.00	0.00	0.00	0.00
Multi-grain cereal for children	QC1 Spike 10 ng/g	9.03	(11.24)	50%	7.71	7.67	5.25	5.20
	"Recovery"	**90%**	**112%**	**45%**	**77%**	**77%**	**53%**	**52%**
Muesli with fruit and nuts	QC2 Blank	0.00	0.12 ^	50%	0.00	0.00	0.00	0.00
Muesli with fruit and nuts	QC2 Spike 10 ng/g	7.66	(10.45)	56%	6.26	6.24	4.45	5.54
	"Recovery"	**77%**	**103%**	**50%**	**63%**	**62%**	**44%**	**55%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 4. Validation results for tropane alkaloids in Muesli (low molecular weight)

Sample Type	Description	Nortropinone	Tropinone	Tropine	Pseudotropine	6-hydroxytropinone	Scopine	Scopoline
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Muesli with fruit and nuts	Blank	0.23 ^	0.00	0.10 ^	0.00	0.00	0.00	0.00
Muesli with fruit and nuts	Spike 1 1ng/g	0.64	0.40	0.77 ^	0.67 ^	0.49	0.55	0.50
Muesli with fruit and nuts	Spike 2 1ng/g	0.64	0.43	0.86 ^	0.62 ^	0.60	0.66	0.51
Muesli with fruit and nuts	Spike 3 1ng/g	0.69	0.45	0.84 ^	0.63 ^	0.67	0.69	0.49
Muesli with fruit and nuts	Spike 4 1ng/g	0.67	0.42	0.87 ^	0.66 ^	0.73	0.70	0.47
Muesli with fruit and nuts	Spike 5 1ng/g	0.70	0.42	0.83 ^	0.61 ^	0.69	0.69	0.50
	Mean	**0.43**	**0.42**	**0.73**	**0.64**	**0.64**	**0.66**	**0.50**
	"Recovery"	**43%**	**42%**	**73%**	**64%**	**64%**	**66%**	**50%**
	RSD	**4%**	**4%**	**5%**	**4%**	**15%**	**9%**	**3%**
Muesli with fruit and nuts	Spike 6 5ng/g	2.47	2.56	4.21 ^	3.60	3.61	3.36	2.86
Muesli with fruit and nuts	Spike 7 5ng/g	2.34	2.59	4.32 ^	3.79	3.68	2.93	2.92
Muesli with fruit and nuts	Spike 8 5ng/g	2.40	2.70	4.23 ^	3.67	3.91	3.05	3.10
Muesli with fruit and nuts	Spike 9 5ng/g	2.40	2.47	4.24 ^	3.56	3.77	3.02	3.03
Muesli with fruit and nuts	Spike 10 5ng/g	2.26	2.57	4.26 ^	3.57	3.80	2.89	2.88
	Mean	**2.14**	**2.58**	**4.15**	**3.64**	**3.75**	**3.05**	**2.96**
	"Recovery"	**43%**	**52%**	**83%**	**73%**	**75%**	**61%**	**59%**
	RSD	**3%**	**3%**	**1%**	**3%**	**3%**	**6%**	**3%**
Muesli with fruit and nuts	MMRS 1 10 ng/g	4.19	6.04	7.86	7.38	7.17	4.59	6.15
Muesli with fruit and nuts	MMRS 2 10 ng/g	4.33	6.60	8.83	8.14	7.88	4.92	6.49
	Mean	**4.03**	**6.32**	**8.24**	**7.76**	**7.52**	**4.76**	**6.32**
	Recovery	**106%**	**82%**	**101%**	**94%**	**100%**	**128%**	**94%**
Cereal	Rikilt QC Blank	0.36 ^	0.00	(0.09) ^	0.00	0.00	0.00	0.00
Cereal	Rikilt QC Spike 10 ng/g	5.92	21.36	13.29	12.21	(14.01)	6.36	11.27
	"Recovery"	**56%**	**214%**	**132%**	**122%**	**140%**	**64%**	**113%**
Multi-grain cereal for children	QC1 Blank	0.27 ^	0.00	(0.09) ^	0.00	0.00	0.00	0.00
Multi-grain cereal for children	QC1 Spike 10 ng/g	(12.35)	6.58	13.23	14.06	(11.74)	(16.34)	11.26
	"Recovery"	**121%**	**66%**	**131%**	**141%**	**117%**	**163%**	**113%**
Wheat bran	QC2 Blank	0.37 ^	0.00	(0.06) ^	0.00	0.00	0.00	0.00
Wheat bran	QC2 Spike 10 ng/g	8.11	3.86	10.82	11.08	(11.82)	(12.65)	8.44
	"Recovery"	**77%**	**39%**	**108%**	**111%**	**118%**	**127%**	**84%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 4 (continued): Validation results for tropane alkaloids in Muesli (Convolamine type)

Sample Type	Description	3a-Phenylacetoxytropane	Convolidine	Fillalbin	Convolvine	Convolamine
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Muesli with fruit and nuts	Blank	0.00	0.00	0.00	0.00	0.00
Muesli with fruit and nuts	Spike 1 1ng/g	0.88	0.56	0.84	0.73	0.92
Muesli with fruit and nuts	Spike 2 1ng/g	0.90	0.55	0.83	0.72	0.91
Muesli with fruit and nuts	Spike 3 1ng/g	0.85	0.54	0.81	0.68	0.87
Muesli with fruit and nuts	Spike 4 1ng/g	0.85	0.58	0.83	0.66	0.88
Muesli with fruit and nuts	Spike 5 1ng/g	0.85	0.56	0.81	0.66	0.84
	Mean	**0.87**	**0.56**	**0.82**	**0.69**	**0.88**
	"Recovery"	**87%**	**56%**	**82%**	**69%**	**88%**
	RSD	**2%**	**3%**	**1%**	**5%**	**3%**
Muesli with fruit and nuts	Spike 6 5ng/g	4.15	2.99	3.96	3.18	4.11
Muesli with fruit and nuts	Spike 7 5ng/g	4.58	3.23	4.21	3.44	4.54
Muesli with fruit and nuts	Spike 8 5ng/g	4.49	3.27	4.21	3.33	4.62
Muesli with fruit and nuts	Spike 9 5ng/g	4.45	3.16	4.06	3.38	4.38
Muesli with fruit and nuts	Spike 10 5ng/g	4.34	3.21	4.03	3.25	4.32
	Mean	**4.40**	**3.17**	**4.09**	**3.31**	**4.39**
	"Recovery"	**88%**	**63%**	**82%**	**66%**	**88%**
	RSD	**4%**	**3%**	**3%**	**3%**	**5%**
Muesli with fruit and nuts	MMRS 1 10 ng/g	8.77	6.11	8.49	6.53	8.64
Muesli with fruit and nuts	MMRS 2 10 ng/g	9.95	6.67	9.36	7.40	9.84
	Mean	**9.36**	**6.39**	**8.92**	**6.96**	**9.24**
	Recovery	**94%**	**99%**	**92%**	**95%**	**95%**
Cereal	Rikilt QC Blank	0.00	0.00	0.00	0.00	0.00
Cereal	Rikilt QC Spike 10 ng/g	8.03	10.19	8.92	6.54	(11.29)
	"Recovery"	**80%**	**102%**	**89%**	**65%**	**113%**
Multi-grain cereal for children	QC1 Blank	0.00	0.00	0.00	0.00	0.00
Multi-grain cereal for children	QC1 Spike 10 ng/g	9.10	12.30	10.16	7.89	9.52
	"Recovery"	**91%**	**123%**	**102%**	**79%**	**95%**
Wheat bran	QC2 Blank	0.00	0.00	0.00	0.00	0.00
Wheat bran	QC2 Spike 10 ng/g	7.64	9.07	8.24	5.47	7.64
	"Recovery"	**76%**	**91%**	**82%**	**55%**	**76%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 4 (continued): Validation results for tropane alkaloids in Muesli (atropine type part 1)

Sample Type	Description	Apoatropine	Noratropine	Homatropine	Aposcopolamine	Norscopolamine	Atropine	Atropine D3
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Rel Resp
Muesli with fruit and nuts	Blank	0.00	0.00	0.00	0.00	0.00	0.00	61%
Muesli with fruit and nuts	Spike 1 1ng/g	0.76	0.29	0.64	0.81	0.67	1.15	65%
Muesli with fruit and nuts	Spike 2 1ng/g	0.86	0.28	0.60	0.81	0.62	1.10	63%
Muesli with fruit and nuts	Spike 3 1ng/g	0.83	0.27	0.58	0.81	0.61	1.37	48%
Muesli with fruit and nuts	Spike 4 1ng/g	0.82	0.27	0.55	0.73	0.58	1.15	56%
Muesli with fruit and nuts	Spike 5 1ng/g	0.82	0.27	0.57	0.77	0.59	1.29	53%
	Mean	**0.82**	**0.27**	**0.59**	**0.79**	**0.61**	**1.21**	**57%**
	"Recovery"	**82%**	**27%**	**59%**	**79%**	**61%**	**121%**	**57%**
	RSD	**4%**	**3%**	**5%**	**5%**	**6%**	**10%**	**12%**
Muesli with fruit and nuts	Spike 6 5ng/g	3.31	1.45	2.84	3.54	2.88	5.40	62%
Muesli with fruit and nuts	Spike 7 5ng/g	4.10	1.47	3.02	4.02	2.96	5.75	61%
Muesli with fruit and nuts	Spike 8 5ng/g	4.16	1.46	2.88	3.93	2.93	5.62	63%
Muesli with fruit and nuts	Spike 9 5ng/g	4.18	1.34	2.75	3.91	2.86	5.54	61%
Muesli with fruit and nuts	Spike 10 5ng/g	4.14	1.42	2.84	3.92	2.75	5.92	57%
	Mean	**3.98**	**1.43**	**2.86**	**3.86**	**2.88**	**5.65**	**61%**
	"Recovery"	**80%**	**29%**	**57%**	**77%**	**58%**	**113%**	**61%**
	RSD	**9%**	**4%**	**3%**	**5%**	**3%**	**4%**	**3%**
Muesli with fruit and nuts	MMRS 1 10 ng/g	9.51	2.28	5.70	7.43	5.44	(10.61)	64%
Muesli with fruit and nuts	MMRS 2 10 ng/g	(10.80)	2.47	6.45	8.40	5.83	(11.25)	68%
	Mean	**10.16**	**2.37**	**6.08**	**7.92**	**5.63**	**10.93**	**66%**
	Recovery	**78%**	**120%**	**94%**	**98%**	**102%**	**103%**	**92%**
Cereal	Rikilt QC Blank	0.00	0.00	0.00	0.00	0.00	0.13	32%
Cereal	Rikilt QC Spike 10 ng/g	(13.63)	3.29	5.16	(10.42)	4.66	(21.51)	31%
	"Recovery"	**136%**	**33%**	**52%**	**104%**	**47%**	**214%**	**32%**
Multi-grain cereal for children	QC1 Blank	0.00	0.00	0.00	0.00	0.00	0.00	67%
Multi-grain cereal for children	QC1 Spike 10 ng/g	8.53	2.49	5.67	8.26	8.39	(10.85)	67%
	"Recovery"	**85%**	**25%**	**57%**	**83%**	**84%**	**108%**	**67%**
Wheat bran	QC2 Blank	0.00	0.00	0.00	0.00	0.00	0.00	37%
Wheat bran	QC2 Spike 10 ng/g	6.00	2.28	4.13	6.47	4.86	(11.00)	47%
	"Recovery"	**60%**	**23%**	**41%**	**65%**	**49%**	**110%**	**37%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 4 (continued): Validation results for tropane alkaloids in Muesli (atropine type part 2)

Sample Type	Description	Littorine	Scopolamine	Scopolamine D3	Anisodamine	Anisodine	2a-OH-methylatropine	o-Acetylscopolamine
		Conc / ng/g	Conc / ng/g	Rel Resp	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Muesli with fruit and nuts	Blank	0.00	0.13 ^	48%	0.00	0.00	0.00	0.00
Muesli with fruit and nuts	Spike 1 1ng/g	0.76	1.38	51%	0.72	0.68	0.38	0.41
Muesli with fruit and nuts	Spike 2 1ng/g	0.72	1.36	48%	0.68	0.64	0.35	0.40
Muesli with fruit and nuts	Spike 3 1ng/g	0.69	1.61	38%	0.66	0.63	0.35	0.39
Muesli with fruit and nuts	Spike 4 1ng/g	0.65	1.41	44%	0.67	0.63	0.36	0.42
Muesli with fruit and nuts	Spike 5 1ng/g	0.67	1.45	42%	0.66	0.62	0.34	0.42
	Mean	**0.70**	**1.31**	**44%**	**0.68**	**0.64**	**0.36**	**0.41**
	"Recovery"	**70%**	**131%**	**44%**	**68%**	**64%**	**36%**	**41%**
	RSD	**6%**	**7%**	**11%**	**4%**	**4%**	**3%**	**2%**
Muesli with fruit and nuts	Spike 6 5ng/g	3.32	6.08	50%	3.26	3.27	1.78	0.87
Muesli with fruit and nuts	Spike 7 5ng/g	3.60	6.38	47%	3.41	3.36	1.85	1.84
Muesli with fruit and nuts	Spike 8 5ng/g	3.53	6.02	48%	3.41	3.38	1.80	2.24
Muesli with fruit and nuts	Spike 9 5ng/g	3.39	5.92	49%	3.28	3.29	1.72	2.27
Muesli with fruit and nuts	Spike 10 5ng/g	3.42	6.42	44%	3.22	3.23	1.73	2.05
	Mean	**3.45**	**6.04**	**48%**	**3.31**	**3.31**	**1.78**	**1.85**
	"Recovery"	**69%**	**121%**	**48%**	**66%**	**66%**	**36%**	**37%**
	RSD	**3%**	**4%**	**5%**	**3%**	**2%**	**3%**	**31%**
Muesli with fruit and nuts	MMRS 1 10 ng/g	6.93	9.12	50%	6.30	6.23	3.06	8.06
Muesli with fruit and nuts	MMRS 2 10 ng/g	8.05	9.80	53%	7.08	7.05	3.31	9.20
	Mean	**7.49**	**9.33**	**51%**	**6.69**	**6.64**	**3.19**	**8.63**
	Recovery	**92%**	**129%**	**93%**	**99%**	**100%**	**112%**	**43%**
Cereal	Rikilt QC Blank	0.00	0.30	19%	(0.01) ^	0.00	0.00	0.00
Cereal	Rikilt QC Spike 10 ng/g	6.54	(23.08)	19%	5.18	4.51	1.98	5.54
	"Recovery"	**65%**	**228%**	**19%**	**52%**	**45%**	**20%**	**55%**
Multi-grain cereal for children	QC1 Blank	0.00	0.00	48%	0.00	0.00	0.00	0.00
Multi-grain cereal for children	QC1 Spike 10 ng/g	8.43	(11.65)	48%	9.06	8.73	3.98	3.79
	"Recovery"	**84%**	**116%**	**48%**	**91%**	**87%**	**40%**	**38%**
Wheat bran	QC2 Blank	0.00	0.00	27%	0.00	0.00	0.00	0.00
Wheat bran	QC2 Spike 10 ng/g	6.04	(11.28)	36%	5.66	5.23	3.26	3.65
	"Recovery"	**60%**	**113%**	**27%**	**57%**	**52%**	**33%**	**37%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 5. Validation results for tropane alkaloids in Bread (low molecular weight)

Sample Type	Description	Nortropinone	Tropinone	Tropine	Pseudotropine	6-hydroxytropinone	Scopine	Scopoline
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Wheat bread	Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Wheat bread	Spike 1 1ng/g	0.35	0.80	0.69	1.12	0.60	0.82	0.80
Wheat bread	Spike 2 1ng/g	0.39	0.61	0.68	0.89	0.62	0.69	0.66
Wheat bread	Spike 3 1ng/g	0.41	0.61	0.69	1.12	0.65	0.84	0.78
Wheat bread	Spike 4 1ng/g	0.40	0.77	0.73	1.39	0.68	1.00	0.92
Wheat bread	Spike 5 1ng/g	0.38	0.71	0.73	1.25	0.60	0.91	0.89
	Mean	**0.39**	**0.70**	**0.70**	**1.15**	**0.63**	**0.85**	**0.81**
	"Recovery"	**39%**	**70%**	**70%**	**115%**	**63%**	**85%**	**81%**
	RSD	**6%**	**13%**	**3%**	**16%**	**6%**	**13%**	**13%**
Wheat bread	Spike 6 5ng/g	1.74	3.63	3.67	6.19	3.07	4.67	4.34
Wheat bread	Spike 7 5ng/g	1.82	3.34	3.83	5.91	3.08	4.46	4.03
Wheat bread	Spike 8 5ng/g	1.83	3.34	3.78	6.01	3.44	4.33	4.16
Wheat bread	Spike 9 5ng/g	1.54	3.23	2.76	6.38	2.46	4.70	4.14
Wheat bread	Spike 10 5ng/g	1.80	3.05	3.78	6.16	3.17	4.77	4.19
	Mean	**1.75**	**3.32**	**3.57**	**6.13**	**3.04**	**4.59**	**4.17**
	"Recovery"	**35%**	**66%**	**71%**	**123%**	**61%**	**92%**	**83%**
	RSD	**7%**	**6%**	**13%**	**3%**	**12%**	**4%**	**3%**
Wheat bread	MMRS 1 10 ng/g	5.17	(11.14)	7.12	(13.12)	8.12	9.44	9.48
Wheat bread	MMRS 2 10 ng/g	5.13	(10.76)	7.09	(12.43)	8.40	9.25	9.12
	Mean	**5.15**	**10.95**	**7.11**	**12.78**	**8.26**	**9.34**	**9.30**
	Recovery	**68%**	**61%**	**100%**	**96%**	**74%**	**98%**	**90%**
Bread	Rikilt QC Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bread	Rikilt QC Spike 10 ng/g	3.71	3.52	6.91	7.25	6.64	5.57	4.82
	"Recovery"	**37%**	**35%**	**69%**	**72%**	**66%**	**56%**	**48%**
Multi-grain bread with seeds	QC1 Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Multi-grain bread with seeds	QC1 Spike 10 ng/g	3.17	6.08	5.57	(11.90)	4.93	8.35	7.49
	"Recovery"	**32%**	**61%**	**56%**	**119%**	**49%**	**83%**	**75%**
White bread	QC2 Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00
White bread	QC2 Spike 10 ng/g	4.22	9.54	7.23	(12.70)	7.64	9.70	9.21
	"Recovery"	**42%**	**95%**	**72%**	**127%**	**76%**	**97%**	**92%**

Table 5 continued: Validation results for tropane alkaloids in Bread (convolamine type)

Sample Type	Description	3a-Phenylacetoxytropane	Convolidine	Fillalbin	Convolvine	Convolamine
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Wheat bread	Blank	(0.02)	0.00	0.00	0.00	(0.19)
Wheat bread	Spike 1 1ng/g	0.78	(0.62)	(0.74)	(0.61)	(0.65)
Wheat bread	Spike 2 1ng/g	0.77	(0.62)	(0.74)	(0.59)	(0.64)
Wheat bread	Spike 3 1ng/g	0.81	(0.63)	(0.77)	(0.59)	(0.66)
Wheat bread	Spike 4 1ng/g	0.84	(0.65)	(0.76)	(0.62)	(0.70)
Wheat bread	Spike 5 1ng/g	0.80	(0.62)	(0.77)	(0.61)	(0.71)
	Mean	**0.80**	**0.63**	**0.76**	**0.60**	**0.67**
	"Recovery"	**80%**	**63%**	**76%**	**60%**	**67%**
	RSD	**3%**	**2%**	**2%**	**2%**	**4%**
Wheat bread	Spike 6 5ng/g	4.07	(2.81)	(3.41)	(2.71)	(3.17)
Wheat bread	Spike 7 5ng/g	3.89	(2.85)	(3.39)	(2.68)	(3.03)
Wheat bread	Spike 8 5ng/g	4.01	(2.81)	(3.40)	(2.73)	(3.10)
Wheat bread	Spike 9 5ng/g	3.06	(2.03)	(2.58)	(2.07)	(2.54)
Wheat bread	Spike 10 5ng/g	4.02	(2.66)	(3.29)	(2.67)	(3.21)
	Mean	**3.81**	**2.63**	**3.21**	**2.57**	**3.01**
	"Recovery"	**76%**	**53%**	**64%**	**51%**	**60%**
	RSD	**11%**	**13%**	**11%**	**11%**	**9%**
Wheat bread	MMRS 1 10 ng/g	8.17	(6.01)	(6.94)	(5.69)	(5.74)
Wheat bread	MMRS 2 10 ng/g	8.31	(5.93)	(6.87)	(5.70)	(5.85)
	Mean	**8.24**	**5.97**	**6.90**	**5.69**	**5.79**
	Recovery	**92%**	**88%**	**93%**	**90%**	**104%**
Bread	Rikilt QC Blank	0.00	0.00	0.00	0.00	0.00
Bread	Rikilt QC Spike 10 ng/g	0.04	(5.91)	(6.74)	(5.75)	(5.36)
	"Recovery"	**No data**	**59%**	**67%**	**57%**	**54%**
Multi-grain bread with seeds	QC1 Blank	(0.02)	0.00	0.00	0.00	(0.11)
Multi-grain bread with seeds	QC1 Spike 10 ng/g	5.74	(4.69)	(5.95)	(4.56)	(6.39)
	"Recovery"	**57%**	**47%**	**60%**	**46%**	**64%**
White bread	QC2 Blank	(0.02)	0.00	0.00	(0.10)	(0.11)
White bread	QC2 Spike 10 ng/g	6.81	(6.48)	(7.56)	(6.06)	(5.56)
	"Recovery"	**68%**	**65%**	**76%**	**61%**	**56%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 5 continued: Validation results for tropane alkaloids in Bread (atropine type part 1)

Sample Type	Description	Apoatropine	Noratropine	Homatropine	Aposcopolamine	Norscopolamine	Atropine	Atropine D3
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Rel Resp
Wheat bread	Blank	(0.09)	0.00	0.00	0.00	0.00	0.00	43%
Wheat bread	Spike 1 1ng/g	(1.12)	0.29	0.38	0.73	0.35	1.11	42%
Wheat bread	Spike 2 1ng/g	(1.09)	0.30	0.39	0.70	0.35	1.36	35%
Wheat bread	Spike 3 1ng/g	(1.14)	0.30	0.39	0.74	0.37	1.35	35%
Wheat bread	Spike 4 1ng/g	(1.17)	0.30	0.40	0.79	0.37	1.13	43%
Wheat bread	Spike 5 1ng/g	(1.16)	0.29	0.40	0.76	0.36	1.23	39%
	Mean	**1.13**	**0.30**	**0.39**	**0.74**	**0.36**	**124%**	**39%**
	"Recovery"	**113%**	**30%**	**39%**	**74%**	**36%**	**124%**	**39%**
	RSD	**3%**	**1%**	**2%**	**4%**	**3%**	**9%**	**10%**
Wheat bread	Spike 6 5ng/g	(5.62)	1.52	1.90	3.79	1.81	6.40	40%
Wheat bread	Spike 7 5ng/g	(5.47)	1.52	1.88	3.55	1.82	5.97	41%
Wheat bread	Spike 8 5ng/g	(5.71)	1.55	1.93	3.90	1.83	6.37	40%
Wheat bread	Spike 9 5ng/g	(4.20)	1.14	1.41	2.80	1.35	5.90	32%
Wheat bread	Spike 10 5ng/g	(5.53)	1.44	1.81	3.84	1.72	5.73	42%
	Mean	**5.30**	**1.43**	**1.79**	**3.58**	**1.71**	**6.08**	**39%**
	"Recovery"	**106%**	**29%**	**36%**	**72%**	**34%**	**122%**	**39%**
	RSD	**12%**	**12%**	**12%**	**13%**	**12%**	**5%**	**10%**
Wheat bread	MMRS 1 10 ng/g	(13.08)	3.07	4.14	7.13	3.62	(12.11)	41%
Wheat bread	MMRS 2 10 ng/g	(13.04)	3.14	4.20	7.20	3.64	(11.95)	41%
	Mean	**13.06**	**3.11**	**4.17**	**7.17**	**3.63**	**12.03**	**41%**
	Recovery	**81%**	**92%**	**86%**	**100%**	**94%**	**101%**	**95%**
Bread	Rikilt QC Blank	0.00	0.00	0.00	0.00	0.00	0.00	49%
Bread	Rikilt QC Spike 10 ng/g	(9.77)	3.07	4.36	6.79	3.68	(10.06)	51%
	"Recovery"	**98%**	**31%**	**44%**	**68%**	**37%**	**101%**	**51%**
Multi-grain bread with seeds	QC1 Blank	0.00	0.00	0.00	0.00	0.00	(0.01)	46%
Multi-grain bread with seeds	QC1 Spike 10 ng/g	(8.94)	2.82	3.45	6.84	3.10	(10.43)	42%
	"Recovery"	**89%**	**28%**	**35%**	**68%**	**31%**	**104%**	**42%**
White bread	QC2 Blank	0.00	0.00	0.00	0.00	0.00	0.00	55%
White bread	QC2 Spike 10 ng/g	(9.19)	3.55	4.62	6.55	4.31	(10.95)	49%
	"Recovery"	**92%**	**36%**	**46%**	**65%**	**43%**	**109%**	**49%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 5 continued: Validation results for tropane alkaloids in Bread (atropine type part 2)

Sample Type	Description	Littorine	Scopolamine	Scopolamine D3	Anisodamine	Anisodine	2a-OH-methylatropine	o-Acetylscopolamine
		Conc / ng/g	Conc / ng/g	Rel Resp	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Wheat bread	Blank	(0.03)	0.00	37%	0.00	0.00	0.00	0.00
Wheat bread	Spike 1 1ng/g	0.36	1.06	36%	0.42	0.41	0.35	0.29
Wheat bread	Spike 2 1ng/g	0.37	1.26	30%	0.43	0.41	0.36	0.28
Wheat bread	Spike 3 1ng/g	0.37	1.25	31%	0.44	0.44	0.37	0.29
Wheat bread	Spike 4 1ng/g	0.38	1.07	37%	0.45	0.45	0.38	0.31
Wheat bread	Spike 5 1ng/g	0.38	1.17	35%	0.45	0.45	0.37	0.31
	Mean	**0.37**	**1.16**	**34%**	**0.44**	**0.43**	**0.37**	**0.29**
	"Recovery"	**37%**	**116%**	**34%**	**44%**	**43%**	**37%**	**29%**
	RSD	**3%**	**8%**	**9%**	**2%**	**4%**	**2%**	**5%**
Wheat bread	Spike 6 5ng/g	1.87	6.07	34%	2.24	2.25	1.92	1.57
Wheat bread	Spike 7 5ng/g	1.75	5.66	36%	2.25	2.24	1.91	1.50
Wheat bread	Spike 8 5ng/g	1.82	6.15	35%	2.25	2.26	1.96	1.34
Wheat bread	Spike 9 5ng/g	1.42	5.59	28%	1.70	1.68	1.44	1.13
Wheat bread	Spike 10 5ng/g	1.89	5.56	35%	2.17	2.14	1.85	1.48
	Mean	**1.75**	**5.81**	**33%**	**2.12**	**2.11**	**1.82**	**1.41**
	"Recovery"	**35%**	**116%**	**33%**	**42%**	**42%**	**36%**	**28%**
	RSD	**11%**	**5%**	**10%**	**11%**	**12%**	**12%**	**12%**
Wheat bread	MMRS 1 10 ng/g	3.85	9.85	35%	4.68	4.44	3.53	5.34
Wheat bread	MMRS 2 10 ng/g	3.94	9.73	36%	4.68	4.42	3.64	5.46
	Mean	**3.90**	**9.79**	**36%**	**4.68**	**4.43**	**3.58**	**5.40**
	Recovery	**90%**	**119%**	**94%**	**91%**	**95%**	**101%**	**52%**
Bread	Rikilt QC Blank	0.00	0.00	41%	0.00	0.00	0.00	0.00
Bread	Rikilt QC Spike 10 ng/g	3.92	9.90	43%	4.70	4.41	0.00	2.89
	"Recovery"	**39%**	**99%**	**43%**	**47%**	**44%**	**No data**	**29%**
Multi-grain bread with seeds	QC1 Blank	0.00	0.00	40%	0.00	0.00	0.00	0.00
Multi-grain bread with seeds	QC1 Spike 10 ng/g	4.00	(10.61)	37%	4.25	3.96	3.37	1.81
	"Recovery"	**40%**	**106%**	**37%**	**43%**	**40%**	**34%**	**18%**
White bread	QC2 Blank	0.00	0.00	51%	0.00	0.00	0.00	0.00
White bread	QC2 Spike 10 ng/g	3.65	(10.82)	44%	5.51	5.34	4.43	2.60
	"Recovery"	**37%**	**108%**	**44%**	**55%**	**53%**	**44%**	**26%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 6. Validation results for tropane alkaloids in Flour (low molecular weight type)

Sample Type	Description	Nortropinone	Tropinone	Tropine	Pseudotropine	6-hydroxytropinone	Scopine	Scopoline
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Buckwheat flour	Blank	0.00	0.00	0.00	0.00	0.00	0.00	(0.05)
Buckwheat flour	Spike 1 1ng/g	< LOD	0.37	0.44	0.39	0.26	0.14	0.31 ^
Buckwheat flour	Spike 2 1ng/g	< LOD	0.39	0.45	0.36	0.24	0.16	0.31 ^
Buckwheat flour	Spike 3 1ng/g	< LOD	0.35	0.42	0.36	0.24	0.14	0.29 ^
Buckwheat flour	Spike 4 1ng/g	< LOD	0.39	0.44	0.39	0.27	0.15	0.30 ^
Buckwheat flour	Spike 5 1ng/g	< LOD	0.39	0.43	0.36	0.26	0.16	0.29 ^
	Mean	**No data**	**0.38**	**0.43**	**0.37**	**0.26**	**0.15**	**0.30**
	"Recovery"	**No data**	**38%**	**43%**	**37%**	**26%**	**15%**	**30%**
	RSD	**No data**	**5%**	**3%**	**4%**	**5%**	**8%**	**3%**
Buckwheat flour	Spike 6 5ng/g	0.10	2.12	2.38	1.74	1.53	1.33	1.42 ^
Buckwheat flour	Spike 7 5ng/g	0.10	1.90	2.27	1.71	1.45	0.91	1.39 ^
Buckwheat flour	Spike 8 5ng/g	0.10	1.97	2.31	1.63	1.48	0.98	1.31 ^
Buckwheat flour	Spike 9 5ng/g	0.11	2.03	2.35	1.76	1.50	1.30	1.36 ^
Buckwheat flour	Spike 10 5ng/g	0.11	1.84	2.31	1.80	1.47	1.04	1.34 ^
	Mean	**0.10**	**1.97**	**2.32**	**1.73**	**1.49**	**1.11**	**1.37**
	"Recovery"	**2%**	**39%**	**46%**	**35%**	**30%**	**22%**	**27%**
	RSD	**5%**	**6%**	**2%**	**4%**	**2%**	**17%**	**3%**
Buckwheat flour	MMRS 1 10 ng/g	0.23	(4.56)	4.18	3.51	2.99	2.79	2.87
Buckwheat flour	MMRS 2 10 ng/g	0.21	(4.47)	4.20	3.41	2.93	1.92	2.87
	Mean	**0.22**	**4.52**	**4.19**	**3.46**	**2.96**	**2.35**	**2.87**
	Recovery	**93%**	**87%**	**111%**	**100%**	**100%**	**94%**	**95%**
Buckwheat flour	Rikilt QC Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Buckwheat flour	Rikilt QC Spike 10 ng/g	0.18	14.35	4.84	8.54	2.71	2.40	3.29
	"Recovery"	**2%**	**143%**	**48%**	**85%**	**27%**	**24%**	**33%**
Millet flour	QC1 Blank	-(0.03) ^	0.00	0.00	(0.07)	0.00	(0.11)	0.00
Millet flour	QC1 Spike 10 ng/g	1.44	1.09	6.94	6.15	3.14	7.25	4.26
	"Recovery"	**14%**	**11%**	**69%**	**61%**	**31%**	**71%**	**43%**
Sorghum flour	QC2 Blank	-(0.07) ^	0.00	0.00	(0.06)	0.00	0.00	0.00
Sorghum flour	QC2 Spike 10 ng/g	3.24	2.79	7.72	7.82	5.86	8.55	5.99
	"Recovery"	**32%**	**28%**	**77%**	**78%**	**59%**	**85%**	**60%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 6 (continued): Validation results for tropane alkaloids in Flour (convolamine type)

Sample Type	Description	3a-Phenylacetoxytropane	Convolidine	Fillalbin	Convolvine	Convolamine
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Buckwheat flour	Blank	(0.04)	0.00	0.00	0.00	(0.18)
Buckwheat flour	Spike 1 1ng/g	0.87	(0.54)	(0.63)	(1.29)	(1.11)
Buckwheat flour	Spike 2 1ng/g	0.86	(0.54)	(0.62)	(1.26)	(1.06)
Buckwheat flour	Spike 3 1ng/g	0.82	(0.50)	(0.59)	(1.26)	(1.07)
Buckwheat flour	Spike 4 1ng/g	0.87	(0.54)	(0.63)	(1.30)	(1.13)
Buckwheat flour	Spike 5 1ng/g	0.88	(0.51)	(0.60)	(1.29)	(1.08)
	Mean	**0.86**	**0.52**	**0.61**	**1.28**	**1.09**
	"Recovery"	**86%**	**52%**	**61%**	**128%**	**109%**
	RSD	**2%**	**4%**	**3%**	**1%**	**3%**
Buckwheat flour	Spike 6 5ng/g	4.04	(2.13)	(2.31)	(5.81)	(4.60)
Buckwheat flour	Spike 7 5ng/g	4.11	(2.14)	(2.32)	(5.96)	(4.77)
Buckwheat flour	Spike 8 5ng/g	4.13	(2.19)	(2.40)	(5.97)	(4.73)
Buckwheat flour	Spike 9 5ng/g	4.08	(2.09)	(2.30)	(5.77)	(4.70)
Buckwheat flour	Spike 10 5ng/g	4.33	(2.19)	(2.43)	(6.29)	(5.27)
	Mean	**4.14**	**2.15**	**2.35**	**5.96**	**4.81**
	"Recovery"	**83%**	**43%**	**47%**	**119%**	**96%**
	RSD	**3%**	**2%**	**2%**	**3%**	**5%**
Buckwheat flour	MMRS 1 10 ng/g	8.17	(4.28)	(4.56)	(12.29)	(10.03)
Buckwheat flour	MMRS 2 10 ng/g	8.10	(4.14)	(4.48)	(11.58)	(9.84)
	Mean	**8.13**	**4.21**	**4.52**	**11.93**	**9.48**
	Recovery	**102%**	**102%**	**104%**	**100%**	**102%**
Buckwheat flour	Rikilt QC Blank	(0.03)	0.00	0.00	0.00	0.00
Buckwheat flour	Rikilt QC Spike 10 ng/g	(0.05)	(4.50)	(5.25)	(13.05)	(11.14)
	"Recovery"	**No data**	**45%**	**53%**	**131%**	**111%**
Millet flour	QC1 Blank	(0.04)	0.00	0.00	0.00	0.00
Millet flour	QC1 Spike 10 ng/g	8.51	(5.63)	(7.57)	(7.93)	(13.18)
	"Recovery"	**85%**	**56%**	**76%**	**79%**	**132%**
Sorghum flour	QC2 Blank	(0.03)	0.00	0.00	0.00	(0.18)
Sorghum flour	QC2 Spike 10 ng/g	8.70	(8.83)	(9.52)	(11.46)	(15.67)
	"Recovery"	**87%**	**88%**	**95%**	**115%**	**157%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 6 (continued): Validation results for tropane alkaloids in Flour (atropine type part 1)

Sample Type	Description	Apoatropine Conc / ng/g	Noratropine Conc / ng/g	Homatropine Conc / ng/g	Aposcopolamine Conc / ng/g	Norscopolamine Conc / ng/g	Atropine Conc / ng/g	Atropine D3 Rel Resp
Buckwheat flour	Blank	(0.14)	0.00	(0.03)	(0.02)	0.00	0.00	64%
Buckwheat flour	Spike 1 1ng/g	(1.45)	0.57	0.67	0.92	0.55	1.16	65%
Buckwheat flour	Spike 2 1ng/g	(1.40)	0.60	0.70	0.90	0.57	1.17	65%
Buckwheat flour	Spike 3 1ng/g	(1.38)	0.54	0.65	0.87	0.53	1.11	68%
Buckwheat flour	Spike 4 1ng/g	(1.48)	0.58	0.69	0.96	0.56	1.23	64%
Buckwheat flour	Spike 5 1ng/g	(1.45)	0.56	0.70	0.92	0.56	1.20	66%
	Mean	**1.43**	**0.57**	**0.68**	**0.91**	**0.55**	**1.17**	**65%**
	"Recovery"	**143%**	**57%**	**68%**	**91%**	**55%**	**117%**	**65%**
	RSD	**3%**	**4%**	**3%**	**4%**	**3%**	**4%**	**2%**
Buckwheat flour	Spike 6 5ng/g	(6.32)	2.88	3.33	4.35	2.85	5.91	66%
Buckwheat flour	Spike 7 5ng/g	(6.38)	2.83	3.28	4.30	2.75	5.75	67%
Buckwheat flour	Spike 8 5ng/g	(6.52)	2.85	3.27	4.53	2.83	6.01	64%
Buckwheat flour	Spike 9 5ng/g	(6.63)	2.85	3.27	4.40	2.77	6.15	62%
Buckwheat flour	Spike 10 5ng/g	(6.98)	2.85	3.35	4.62	2.63	5.66	70%
	Mean	**6.57**	**2.85**	**3.30**	**4.44**	**2.77**	**5.90**	**66%**
	"Recovery"	**131%**	**57%**	**66%**	**89%**	**55%**	**118%**	**66%**
	RSD	**4%**	**1%**	**1%**	**3%**	**3%**	**3%**	**5%**
Buckwheat flour	MMRS 1 10 ng/g	(14.45)	5.39	6.90	8.15	5.12	(11.50)	65%
Buckwheat flour	MMRS 2 10 ng/g	(13.90)	5.41	7.03	8.10	5.05	(11.98)	62%
	Mean	**14.17**	**5.40**	**6.97**	**8.12**	**5.08**	**11.74**	**64%**
	Recovery	**93%**	**106%**	**95%**	**109%**	**109%**	**100%**	**103%**
Buckwheat flour	Rikilt QC Blank	0.00	0.00	0.00	0.00	0.00	0.00	63%
Buckwheat flour	Rikilt QC Spike 10 ng/g	(5.88)	5.62	7.13	3.30	5.19	(10.87)	72%
	"Recovery"	**59%**	**56%**	**71%**	**33%**	**52%**	**109%**	**72%**
Millet flour	QC1 Blank	(0.14)	0.00	0.00	0.00	0.00	0.00	38%
Millet flour	QC1 Spike 10 ng/g	(15.39)	3.72	3.21	6.43	3.63	(11.91)	38%
	"Recovery"	**154%**	**37%**	**32%**	**64%**	**36%**	**119%**	**38%**
Sorghum flour	QC2 Blank	0.00	(0.02)	0.00	0.00	0.00	0.00	54%
Sorghum flour	QC2 Spike 10 ng/g	(18.29)	5.45	4.19	8.85	6.23	(11.64)	50%
	"Recovery"	**183%**	**55%**	**42%**	**88%**	**62%**	**116%**	**50%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 6 (continued): Validation results for tropane alkaloids in Flour (atropine type part 2)

Sample Type	Description	Littorine	Scopolamine	Scopolamine D3	Anisodamine	Anisodine	2a-OH-methylatropine	o-Acetylscopolamine
		Conc / ng/g	Conc / ng/g	Rel Resp	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Buckwheat flour	Blank	(0.04)	0.00	69%	(0.01)	0.00	0.00	0.00
Buckwheat flour	Spike 1 1ng/g	0.71	1.11	70%	0.66	0.65	0.65	0.67
Buckwheat flour	Spike 2 1ng/g	0.72	1.14	71%	0.68	0.67	0.67	0.65
Buckwheat flour	Spike 3 1ng/g	0.69	1.08	72%	0.64	0.63	0.62	0.65
Buckwheat flour	Spike 4 1ng/g	0.74	1.17	69%	0.66	0.64	0.66	0.67
Buckwheat flour	Spike 5 1ng/g	0.72	1.16	71%	0.65	0.63	0.67	0.66
	Mean	**0.72**	**1.13**	**71%**	**0.66**	**0.64**	**0.65**	**0.66**
	"Recovery"	**72%**	**113%**	**71%**	**66%**	**64%**	**65%**	**66%**
	RSD	**3%**	**3%**	**2%**	**2%**	**2%**	**3%**	**2%**
Buckwheat flour	Spike 6 5ng/g	3.48	5.52	72%	3.32	3.26	3.32	3.30
Buckwheat flour	Spike 7 5ng/g	3.40	5.43	71%	3.20	3.20	3.19	3.16
Buckwheat flour	Spike 8 5ng/g	3.39	5.59	70%	3.23	3.18	3.26	3.11
Buckwheat flour	Spike 9 5ng/g	3.43	5.80	68%	3.16	3.11	3.23	3.27
Buckwheat flour	Spike 10 5ng/g	3.46	5.42	75%	3.20	3.15	3.26	3.26
	Mean	**3.43**	**5.55**	**71%**	**3.22**	**3.18**	**3.25**	**3.22**
	"Recovery"	**69%**	**111%**	**71%**	**64%**	**64%**	**65%**	**64%**
	RSD	**1%**	**3%**	**3%**	**2%**	**2%**	**1%**	**2%**
Buckwheat flour	MMRS 1 10 ng/g	7.25	9.39	68%	6.34	5.93	6.10	9.75
Buckwheat flour	MMRS 2 10 ng/g	7.44	9.87	67%	6.43	5.81	6.37	9.77
	Mean	**7.35**	**9.63**	**68%**	**6.39**	**5.87**	**6.23**	**9.76**
	Recovery	**93%**	**115%**	**105%**	**101%**	**108%**	**104%**	**119%**
Buckwheat flour	Rikilt QC Blank	0.00	0.00	68%	0.01	0.00	0.00	0.00
Buckwheat flour	Rikilt QC Spike 10 ng/g	7.72	(12.24)	72%	6.68	6.45	0.00	3.94
	"Recovery"	**77%**	**122%**	**72%**	**67%**	**65%**	**No data**	**39%**
Millet flour	QC1 Blank	0.00	0.00	31%	0.01	0.00	0.00	0.00
Millet flour	QC1 Spike 10 ng/g	5.32	(10.97)	32%	4.87	4.66	4.10	4.59
	"Recovery"	**53%**	**110%**	**32%**	**49%**	**47%**	**41%**	**46%**
Sorghum flour	QC2 Blank	0.00	0.00	42%	0.01	0.00	0.00	0.00
Sorghum flour	QC2 Spike 10 ng/g	6.21	(10.96)	39%	7.23	6.96	5.85	5.83
	"Recovery"	**62%**	**110%**	**39%**	**72%**	**70%**	**58%**	**58%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 7. Validation results for tropane alkaloids in cookie (low molecular weight type)

Sample Type	Description	Nortropinone	Tropinone	Tropine	Pseudotropine	6-hydroxytropinone	Scopine	Scopoline
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Bicuits with fruit	Blank	0.00	0.00	0.00	0.11	0.00	0.00	0.00
Bicuits with fruit	Spike 1 1ng/g	0.37	0.40	0.53	0.57	0.57	0.62	0.45
Bicuits with fruit	Spike 2 1ng/g	0.32	0.36	0.56	0.60	0.57	0.62	0.44
Bicuits with fruit	Spike 3 1ng/g	0.30	0.33	0.50	0.56	0.48	0.58	0.43
Bicuits with fruit	Spike 4 1ng/g	0.35	0.38	0.56	0.59	0.55	0.61	0.44
Bicuits with fruit	Spike 5 1ng/g	0.37	0.39	0.48	0.54	0.56	0.61	0.46
	Mean	**0.34**	**0.37**	**0.53**	**0.46**	**0.55**	**0.61**	**0.44**
	"Recovery"	**34%**	**37%**	**53%**	**46%**	**55%**	**61%**	**44%**
	RSD	**9%**	**7%**	**7%**	**4%**	**7%**	**3%**	**3%**
Bicuits with fruit	Spike 6 5ng/g	1.48	2.01	2.84	2.53	2.67	3.36	2.31
Bicuits with fruit	Spike 7 5ng/g	1.44	2.07	2.82	2.59	2.55	3.07	2.25
Bicuits with fruit	Spike 8 5ng/g	1.64	2.26	3.02	2.53	2.93	3.37	2.40
Bicuits with fruit	Spike 9 5ng/g	1.43	1.81	2.86	2.49	2.53	3.07	2.18
Bicuits with fruit	Spike 10 5ng/g	1.47	1.88	2.85	2.52	2.55	3.12	2.16
	Mean	**1.49**	**2.01**	**2.88**	**2.42**	**2.65**	**3.20**	**2.26**
	"Recovery"	**30%**	**40%**	**58%**	**48%**	**53%**	**64%**	**45%**
	RSD	**6%**	**9%**	**3%**	**2%**	**6%**	**5%**	**4%**
Bicuits with fruit	MMRS 1 10 ng/g	4.25	8.86	8.10	6.51	7.70	8.00	7.11
Bicuits with fruit	MMRS 2 10 ng/g	4.06	8.72	7.76	6.31	7.55	7.72	7.13
	Mean	**4.15**	**8.79**	**7.93**	**6.30**	**7.62**	**7.86**	**7.12**
	Recovery	**72%**	**46%**	**73%**	**77%**	**69%**	**81%**	**63%**
Cookie	Rikilt QC Blank	0.06	0.00	0.00	0.15	0.00	0.00	0.00
Cookie	Rikilt QC Spike 10 ng/g	7.11	16.54	14.79	11.38	13.13	14.28	11.26
	"Recovery"	**35%**	**83%**	**74%**	**56%**	**66%**	**71%**	**56%**
Sweet plain biscuits	QC1 Blank	0.00	0.00	0.00	0.17	0.12	0.00	0.00
Sweet plain biscuits	QC1 Spike 10 ng/g	2.84	2.26	6.68	5.22	6.12	6.36	4.22
	"Recovery"	**28%**	**23%**	**67%**	**51%**	**60%**	**64%**	**42%**
Biscuits for children	QC2 Blank	0.20	0.00	0.01	0.13	0.09	0.00	0.00
Biscuits for children	QC2 Spike 10 ng/g	1.97	2.57	4.53	4.14	3.10	3.94	3.37
	"Recovery"	**18%**	**26%**	**45%**	**40%**	**30%**	**39%**	**34%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 7 (continued): Validation results for tropane alkaloids in cookie (convolamine type)

Sample Type	Description	3a-Phenylacetoxytropane	Convolidine	Fillalbin	Convolvine	Convolamine
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Bicuits with fruit	Blank	0.01	0.00	0.00	0.00	0.05
Bicuits with fruit	Spike 1 1ng/g	0.82	(0.67)	(1.04)	0.74	0.94
Bicuits with fruit	Spike 2 1ng/g	0.81	(0.65)	(1.01)	0.68	0.91
Bicuits with fruit	Spike 3 1ng/g	0.77	(0.54)	(0.95)	0.68	0.90
Bicuits with fruit	Spike 4 1ng/g	0.81	(0.63)	(1.03)	0.68	0.90
Bicuits with fruit	Spike 5 1ng/g	0.80	(0.63)	(1.00)	0.74	0.95
	Mean	**0.79**	**0.62**	**1.01**	**0.71**	**0.92**
	"Recovery"	**79%**	**62%**	**101%**	**71%**	**92%**
	RSD	**2%**	**8%**	**3%**	**5%**	**2%**
Bicuits with fruit	Spike 6 5ng/g	4.04	(2.77)	(4.70)	3.39	4.39
Bicuits with fruit	Spike 7 5ng/g	4.12	(2.76)	(4.72)	3.54	4.51
Bicuits with fruit	Spike 8 5ng/g	4.11	(2.87)	(4.80)	3.24	4.33
Bicuits with fruit	Spike 9 5ng/g	3.94	(2.81)	(4.63)	3.38	4.37
Bicuits with fruit	Spike 10 5ng/g	3.95	(2.69)	(4.66)	3.23	4.21
	Mean	**4.02**	**2.78**	**4.70**	**3.36**	**4.36**
	"Recovery"	**80%**	**56%**	**94%**	**67%**	**87%**
	RSD	**2%**	**2%**	**1%**	**4%**	**2%**
Bicuits with fruit	MMRS 1 10 ng/g	(10.53)	(6.98)	(11.37)	9.81	(11.55)
Bicuits with fruit	MMRS 2 10 ng/g	(10.17)	(6.78)	(11.39)	8.26	(10.62)
	Mean	**10.34**	**6.88**	**11.38**	**9.04**	**11.08**
	Recovery	**78%**	**81%**	**83%**	**74%**	**79%**
Cookie	Rikilt QC Blank	0.00	0.00	0.00	0.00	0.00
Cookie	Rikilt QC Spike 10 ng/g	9.76	(13.96)	(20.75)	(18.57)	(20.76)
	"Recovery"	**98%**	**70%**	**104%**	**93%**	**104%**
Sweet plain biscuits	QC1 Blank	0.01	0.00	0.00	0.00	0.00
Sweet plain biscuits	QC1 Spike 10 ng/g	9.45	(5.38)	(9.64)	7.54	(10.28)
	"Recovery"	**94%**	**54%**	**96%**	**75%**	**103%**
Biscuits for children	QC2 Blank	0.00	0.00	0.00	0.00	0.00
Biscuits for children	QC2 Spike 10 ng/g	8.64	(3.09)	(4.64)	4.38	8.40
	"Recovery"	**86%**	**31%**	**46%**	**44%**	**84%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Table 7 (continued): Validation results for tropane alkaloids in cookie (atropine type part 1)

Sample Type	Description	Apoatropine	Noratropine	Homatropine	Aposcopolamine	Norscopolamine	Atropine	Atropine D3
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Rel Resp
Bicuits with fruit	Blank	0.05	0.00	0.00	0.00	0.00	0.00	60%
Bicuits with fruit	Spike 1 1ng/g	0.80	0.61	0.62	0.67	0.70	1.13	60%
Bicuits with fruit	Spike 2 1ng/g	0.81	0.59	0.58	0.64	0.68	1.13	57%
Bicuits with fruit	Spike 3 1ng/g	0.73	0.57	0.60	0.60	0.66	1.17	56%
Bicuits with fruit	Spike 4 1ng/g	0.80	0.58	0.60	0.65	0.69	1.19	56%
Bicuits with fruit	Spike 5 1ng/g	0.80	0.59	0.60	0.65	0.74	1.15	59%
	Mean	**0.79**	**0.59**	**0.60**	**0.64**	**0.69**	**1.15**	**58%**
	"Recovery"	**79%**	**59%**	**60%**	**64%**	**69%**	**115%**	**58%**
	RSD	**4%**	**3%**	**2%**	**4%**	**4%**	**2%**	**3%**
Bicuits with fruit	Spike 6 5ng/g	4.01	2.94	2.99	3.34	3.39	5.96	57%
Bicuits with fruit	Spike 7 5ng/g	3.98	2.96	3.09	3.43	3.44	6.04	58%
Bicuits with fruit	Spike 8 5ng/g	3.87	2.77	2.80	3.30	3.37	5.79	56%
Bicuits with fruit	Spike 9 5ng/g	3.78	2.90	2.88	3.20	3.25	6.20	53%
Bicuits with fruit	Spike 10 5ng/g	3.93	2.77	2.80	3.18	3.36	5.78	56%
	Mean	**3.91**	**2.87**	**2.91**	**3.29**	**3.36**	**5.95**	**56%**
	"Recovery"	**78%**	**57%**	**58%**	**66%**	**67%**	**119%**	**56%**
	RSD	**2%**	**3%**	**4%**	**3%**	**2%**	**3%**	**3%**
Bicuits with fruit	MMRS 1 10 ng/g	(12.50)	7.51	7.57	9.05	7.81	(13.84)	63%
Bicuits with fruit	MMRS 2 10 ng/g	(12.55)	6.75	6.70	8.72	7.30	(13.77)	55%
	Mean	**12.52**	**7.13**	**7.13**	**8.88**	**7.56**	**13.81**	**59%**
	Recovery	**63%**	**80%**	**82%**	**74%**	**89%**	**86%**	**95%**
Cookie	Rikilt QC Blank	0.00	0.00	0.00	0.00	0.00	0.00	78%
Cookie	Rikilt QC Spike 10 ng/g	(16.76)	(14.46)	(15.96)	(12.86)	(15.28)	(20.06)	80%
	"Recovery"	**84%**	**72%**	**80%**	**64%**	**76%**	**100%**	**80%**
Sweet plain biscuits	QC1 Blank	0.00	0.01	0.00	0.00	0.00	0.00	61%
Sweet plain biscuits	QC1 Spike 10 ng/g	8.11	6.32	6.42	5.90	6.67	(12.74)	58%
	"Recovery"	**81%**	**63%**	**64%**	**59%**	**67%**	**127%**	**58%**
Biscuits for children	QC2 Blank	0.00	0.00	0.00	0.00	0.00	0.00	43%
Biscuits for children	QC2 Spike 10 ng/g	7.33	3.30	3.71	4.92	2.99	(12.34)	43%
	"Recovery"	**73%**	**33%**	**37%**	**49%**	**30%**	**123%**	**43%**

Table 7 (continued): Validation results for tropane alkaloids in cookie (atropine type part 2)

Sample Type	Description	Littorine	Scopolamine	Scopolamine D3	Anisodamine	Anisodine	2a-OH-methylatropine	o-Acetylscopolamine
		Conc / ng/g	Conc / ng/g	Rel Resp	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Bicuits with fruit	Blank	0.00	0.03	53%	0.00	0.00	0.00	0.00
Bicuits with fruit	Spike 1 1ng/g	0.64	1.17	57%	0.77	0.80	0.73	0.38
Bicuits with fruit	Spike 2 1ng/g	0.60	1.09	58%	0.77	0.77	0.72	0.40
Bicuits with fruit	Spike 3 1ng/g	0.65	1.19	55%	0.72	0.74	0.70	0.36
Bicuits with fruit	Spike 4 1ng/g	0.62	1.25	52%	0.76	0.78	0.72	0.35
Bicuits with fruit	Spike 5 1ng/g	0.64	1.20	55%	0.73	0.77	0.70	0.41
	Mean	**0.63**	**1.15**	**55%**	**0.75**	**0.77**	**0.71**	**0.38**
	"Recovery"	**63%**	**115%**	**55%**	**75%**	**77%**	**71%**	**38%**
	RSD	**3%**	**5%**	**4%**	**3%**	**3%**	**2%**	**8%**
Bicuits with fruit	Spike 6 5ng/g	3.01	5.88	56%	3.73	3.92	3.54	2.00
Bicuits with fruit	Spike 7 5ng/g	3.23	6.26	54%	3.80	3.98	3.55	1.95
Bicuits with fruit	Spike 8 5ng/g	2.85	5.85	52%	3.73	3.91	3.37	2.00
Bicuits with fruit	Spike 9 5ng/g	3.01	6.43	50%	3.70	3.87	3.51	1.95
Bicuits with fruit	Spike 10 5ng/g	2.80	5.79	53%	3.53	3.75	3.34	1.95
	Mean	**2.98**	**6.01**	**53%**	**3.70**	**3.89**	**3.46**	**1.97**
	"Recovery"	**60%**	**120%**	**53%**	**74%**	**78%**	**69%**	**39%**
	RSD	**6%**	**5%**	**4%**	**3%**	**2%**	**3%**	**1%**
Bicuits with fruit	MMRS 1 10 ng/g	8.02	(12.21)	57%	8.87	9.00	8.52	(10.44)
Bicuits with fruit	MMRS 2 10 ng/g	7.06	(12.04)	51%	8.64	8.71	7.98	9.51
	Mean	**7.54**	**12.09**	**54%**	**8.76**	**8.86**	**8.25**	**9.98**
	Recovery	**79%**	**99%**	**98%**	**85%**	**88%**	**84%**	**40%**
Cookie	Rikilt QC Blank	0.00	0.00	76%	0.00	0.00	0.00	0.00
Cookie	Rikilt QC Spike 10 ng/g	(16.38)	(17.06)	75%	(14.55)	(16.93)	8.61	(15.91)
	"Recovery"	**82%**	**85%**	**75%**	**73%**	**85%**	**86%**	**80%**
Sweet plain biscuits	QC1 Blank	0.00	0.00	62%	0.00	0.00	0.00	0.00
Sweet plain biscuits	QC1 Spike 10 ng/g	7.08	(12.26)	55%	7.75	8.09	7.68	6.49
	"Recovery"	**71%**	**123%**	**55%**	**78%**	**81%**	**77%**	**65%**
Biscuits for children	QC2 Blank	0.00	0.00	35%	0.00	0.00	0.00	0.00
Biscuits for children	QC2 Spike 10 ng/g	6.07	(10.15)	35%	4.16 ^	4.34	3.75	6.19
	"Recovery"	**61%**	**101%**	**35%**	**42%**	**43%**	**37%**	**62%**

Annex 3. Fera Validation report for tropane alkaloids in cereal products

Appendix I:

Protocol for the validation of LC-MS/MS analysis of TAs in breakfast cereals, plain flours, bread and cookies

Patrick Mulder, Version 1, October 2015

- 1. This document describes the validation procedure for in-house validation of the analysis of tropane alkaloids in breakfast cereals, plain flours, bread and cookies at: 1, 5 and 25 ng/g (MMS range: 0-50 ng/ml).

Part A: Samples and matrices

- 2. Validation series: breakfast cereals (3 series), plain flours (1 series), bread (1 series), cookies (1 series).

  Full validation (3 series) is carried out for breakfast cereals, the other matrices are additionally validated (1 series).

- 3. Samples selected:

Matrix	Product 1	Product 2	Product 3
Breakfast cereals	Multi-grain based cereal for young children	Wheat bran flakes for breakfast	Muesli with fruit and nuts
Plain flours	Buckwheat flour	Millet flour*	Sorghum flour*
Bread	Wheat bread	Multi-grain bread with seeds*	White bread*
Cookies	Biscuits with fruit	Sweat plain biscuits*	Biscuits for children*

- Products listed with an * are included as additional QCs in the validation of the method (when validating product 1). For breakfast samples product 2 and 3 are used as QCs when validating product 1; etc.
- Products listed under 1 have also been used to prepare QC samples for inter-laboratory comparison and quality control when running sample series for EFSA. Products listed under 1 can also be used as blank material for MMS/MMRS samples when running sample series for EFSA.

Part B: Standards, solutions and equipment

4. Mixed standard solutions of TAs (100 and 1000 ng/ml) in methanol

- Prepare mixed standard solutions of TAs as described in the SOP for LC-MS/MS analysis of TAs in cereals and cereal products.
- The solutions are stored at -20ºC in the dark for the duration of the project.

5. Mixed internal standard solution of atropine-d3 and scopolamine-d3 (1000 ng/ml) in methanol

- Prepare mixed internal standard solutions of atropine-d3 and scopolamine-d3 as described in the SOP for LC-MS/MS analysis of TAs in cereals and cereal products.
- The solution is stored at -20^{o}C in the dark for the duration of the project.

6. Extraction, SPE and HPLC solutions

- Prepare extraction, SPE and HPLC solutions as as described in the SOP for LC-MS/MS analysis of TAs in cereals and cereal products.
- Store all solutions at room temperature.

7. Equipment

- Use the same equipment as described in in the SOP for LC-MS/MS analysis of TAs in cereals and cereal products.

Part C: Extraction and sample preparation procedure

- Samples should be finely ground and homogenized.
- Test sample size: 4 g.

8. Spiking levels for validation

- 6 samples spiked with 1 ng/g (= 40 µl of 100 ng/ml std TA mix).
- 6 samples spiked with 5 ng/g (= 200 µl of 100 ng/ml std TA mix).
- 6 samples spiked with 25 ng/g (= 100 µl of 1000 ng/ml std TA mix).

9. MMS, MMRS and QC samples

- 8 Matrix matched standards (MMS): spike the blank material with 0 - 50 ng/g TA std mix with a positive displacement pipet. See Table:

MMS (ng/g)	TA std mix 100 ng/ml (µL)	TA std mix 1000 ng/ml (µL)
0	-	-
0.5	20	-
1	40	-
2.5	100	-
5	200	-
10	-	40
25	-	100
50	-	200

- 2 MMRS samples: a blank sample spiked in duplicate after clean up with 10 ng/g (= 10 µl of 1000 ng/ml std mix): process 2 aliquots of 10 ml of the blank sample.
- 4 independent QC samples: 2 different blank samples (see table and these spiked at 10 ng/ml (= 75 µl of 100 ng/ml).
- Mix samples by vortexing and let stand for 30 min.

10. Extraction

- To all samples: add 10 ng/g IS (= 40 µl of IS solution 1000 ng/ml with a positive displacement pipet. Mix sample by vortexing and let stand for 30 min.
- Add 40 ml of extraction solution to the tubes by using a dispenser.
- Extract for 30 min on a rotary tumbler (overhead shaker).
- Centrifuge for 15 min at 3500 rpm.
- Take 10 ml extract for SPE purification.

11. Solid phase extraction
    - SPE cartridge: OASIS MCX 150 mg/6cc or Strata X-C, 200 mg/6 ml.
    - Condition cartridge with 6 ml methanol.
    - Equilibrate cartridge with 6 ml of 1% formic acid solution.
    - Apply **10 ml** of extract to the cartridge.
    - Wash cartridge with 6 ml of 1% formic acid solution.
    - Dry cartridge under reduced pressure.
    - Elute cartridge with 6 ml of 0.25% ammonia in methanol and collect eluates in 10 ml PP tubes with screw cap.
    - Evaporate to dryness under a nitrogen atmosphere in a water bath of 50ºC using a Turbovap.

12. Preparation of extracts for LC-MS/MS analysis.
    - Dissolve the dry residue in 500 µl of sample extract solvent and vortex for 15 s.
    - To the 2 MMRS extracts, 10 µl of a TA std mix solution (1000 ng/ml TA std mix) in methanol is added and 490 µl water.
    - Transfer extracts to 500 µl filtervials and close.
    - Store vials containing the final extracts can be stored in the freezer until analysis.

Part D: LC-MS/MS analysis

- Waters Xevo TQ-S. Tune method: SOPA1061; Inlet method: TAs_EFSA_2015; MS method: TAs_EFSA_2015.
- Details of the LC-MS/MS analysis are described in the SOP for LC-MS/MS analysis of TAs in cereals and cereal products.
- In total each validation series contains: 8 MMS, 2 MMRS, 4 QC, 18 spiked samples = 32 samples.

13. Injection order
    - WS (2-3x)
    - Blank (1)
    - MMS (8)
    - MMRS (2)
    - Blank (1)
    - QCs (4)
    - Blank (1)
    - Samples (18)
    - Blank (1)
    - MMS (8)
    - In total ca. 45 injections per validation series.

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REPORT

Tropane Alkaloids in Tea – EFSA Survey

Validation Report of RIKILT Method

FS 102116

July 2016

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Annex 4. Fera Validation report for tropane alkaloids in teas

1 Introduction

This report describes the procedure and the performance characteristics of a test method for the LC-MS analysis of 24 tropane alkaloids in tea. The tropane alkaloids analysed using this method are 6-hydroxytropinone, nortropinone, pseudotropine, scopine, scopoline, tropine, tropinone, acetylscopolamine, anisodamine, anisodine, apoatropine, aposcopolamine, atropine, convolamine, convolidine, convolvine, fillalbin, homatropine, α-hydroxymethylatropine, littorine, noratropine, norscopolamine, phenylacetoxytropane and scopolamine. D_3-atropine and D_3-scopolamine are used as internal standards.

Validation of the method was performed by the analysis of tea with six replicate spikes at three concentrations (1 ng/g, 5 ng/g and 25 ng/g), quantified using calibration standards prepared in solvent. The matrix used for the validation was green tea supplied by Rikilt. Three additional types of tea – peppermint, fennel and black tea – were included as QC spikes at 10 ng/g, and green tea spiked at 10 ng/g was provided by Rikilt. Recovery was assessed via the inclusion of extracts spiked after extraction and clean-up at a concentration equivalent to 10 ng/g.

The limit of detection (LOD) of the method was defined as the concentration of each analyte required to give a chromatographic peak with signal to noise (s:n) ratio ≥ 3. This was assessed by extrapolation from the s:n ratio of each analyte in the lowest calibration standard and then applying a correction for recovery to determine the LOD in matrix. The limit of quantification (LOQ) of the method was defined as the concentration of the lowest calibration standard with s:n ≥ 3 and with a peak area fitting a calibration line with residuals ≤ 30 % and $r^2 \geq 0.95$, again with a recovery correction applied to obtain the LOQ in matrix.

2 Materials and Methods

Sample extraction, clean-up and analysis were performed following *Standard Operation Protocol for LC-MS/MS analysis of TAs in cereals, cereal products, (herbal) dry tea, (herbal) tea infusions and stir-fry vegetable products, SOP for EFSA project Occurrence of TAs in food, Patrick Mulder & Patricia López Sánchez, February 2016, version g* (see Annex 2). The changes that were made to the SOP that are relevant for analysis of teas are highlighted in red text. Any deviations from this SOP are recorded below. The same SOP was also used for analysis of green beans and mixed vegetables, due to the small number of samples analysed it was not required to carry out validation for these matrices, instead each sample was analysed with a single point standard addition (overspike).

2.1 Standards

Stock solutions of the individual tropane alkaloids at 100 µg/mL were provided by Rikilt. A mixed standard at 1000 ng/mL was prepared by pipetting 200 µL of each stock into a 20 mL volumetric flask and making up to the mark with methanol. This mixed standard was then further diluted to 100 ng/mL by pipetting 1 mL into a 10 mL volumetric flask and making up to the mark with methanol and to 10 ng/mL by pipetting 100 µL into a 10 mL volumetric flask and making up to the mark with methanol.

Stock solutions of the individual internal standards at 100 µg/mL were also provided by Rikilt. A mixed internal standard solution at 1000 ng/mL was prepared by pipetting 250 µL of each stock into a 25 mL volumetric flask and making up to the mark with methanol.

This method of preparing the mixed standard solutions differs from the SOP (see Annex 2 sections 1.2-2.2).

2.2 Reagents

All reagents used were of an equivalent quality to that specified in the SOP (Appendix I section 3) and mixed solvents were prepared in the same way (scaling up or down for quantity required). The elution solvent used was 1 % ammonia in methanol, which was prepared by dilution of 7 N ammonia in methanol (21 mL 7 N ammonia in methanol was mixed with 229 mL methanol). This method of preparation differs from the SOP (Appendix I section 3.2.4).

2.3 Equipment

The equipment used was equivalent to that specified in the SOP (Appendix I section 4) with the following exceptions:

An orbital shaker (AQS) was used instead of an overhead shaker.

Samples were dried under nitrogen on a dry block sample concentrator, not in a TurboVap.

Samples were collected after elution and dried down in glass sample tubes instead of polypropylene.

Samples were filtered using 0.2 µm nylon syringe filters into glass vials instead of using 0.45 µm polypropylene PTFE filtervials. A smaller particle filter was required to use with the UPLC column and it was found that the samples would not pass through 0.2 µm PTFE filters, possibly due to swelling caused by the aqueous solvent.

The SPE cartridges chosen were Phenomenex Strata-X-C, 33 µm, 200 mg, 6 mL.

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2.4 Sample Extraction and Clean-Up

Sample extraction and clean-up was performed following the SOP (Annex 2 section 6), with the changes to equipment noted above.

The validation batch consisted of green tea (blank material supplied by Rikilt) spiked at 1 ng/g, 5 ng/g and 25 ng/g. Six replicates were prepared at each spike level and the blank material was also analysed. QC samples of peppermint, fennel and black tea were spiked at 10 ng/g and the blank materials were also analysed. Green tea spiked at 10 ng/g was provided by Rikilt. This information is summarised in Table 1.

Calibration standards were prepared in solvent in a range equivalent to 0.1-50 ng/g (0.1-50 ng/mL in the vial). The use of calibration standards prepared in solvent deviates from the SOP, which specifies the use of matrix extracted standards.

Table 1: Matrices used for validation spikes and QC in tea validation batch.

Validation Matrix	QC1	QC2	QC3	Rikilt QC
Green tea Rikilt	Peppermint tea S16-002253	Fennel tea S16-002254	Black tea S16-002255	Green tea Rikilt

The batch was weighed, spiked and extracted on one day, and the extracts collected after centrifugation and stored in the fridge overnight before clean-up. After SPE clean-up, evaporation and reconstitution the samples were refrigerated prior to analysis by LC-MS. They were not stored in the freezer as specified in the SOP (Annex 2 section 6.4) due to concerns about expansion of the aqueous sample in the insert vial on freezing.

Matrix-matched recovery samples (MMRS) were prepared by taking two additional aliquots of the blank green tea through the clean-up and evaporation procedure and then adding 450 µL water and 50 µL of the tropane alkaloid mixed standard at 100 ng/mL. This method differs from the SOP (Annex 2 section 6.4).

2.5 LC-MS Analysis

Samples were analysed using an Acquity UPLC system coupled to a Xevo TQ-S tandem quadrupole mass spectrometer (Waters).

2.5.1 LC Method

Column: Acquity BEH C_{18} 1.7 µm (150 x 2.1 mm) (Waters)

Column temperature: 50 °C

Injection volume: 2 µL

Mobile phase A: 10 mM ammonium carbonate in water

Mobile phase B: Acetonitrile

Gradient timetable:

Time / min	% B	Flow Rate
0.0	0	0.40
2.0	0	0.40
12.0	60	0.40
12.2	99	0.55
15.0	99	0.55
16.0	0	0.55
19.0	0	0.55
19.1	0	0.40
20.0	0	0.40

The gradient profile differs from that in the SOP (Annex 2 section 8.2.2) by the addition of the flush step at 99 % B.

2.5.2 MS Method

Ionisation mode: Electrospray positive

Capillary voltage: 1 kV

Desolvation temperature: 500 °C

Desolvation gas flow rate: 1000 L/h

Nebuliser gas flow: 7 bar

Source temperature: 150 °C

Cone gas flow rate: 100 L/h

Collision gas flow rate: 0.15 mL/min (3.5 mbar)

Resolution: Unit mass

MRM conditions: As SOP (Annex 2 section 7.2.3)

These source tune parameters differ slightly from those in the SOP (Appendix I section 8.2.1)

3 Results and Discussion

Chromatographic peak shapes were generally good, although peak tailing was observed for some analytes (particularly tropine, scopoline, convolidine and fillalbin). Compounds with the same m/z were chromatographically well resolved.

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The response for the majority of analytes was generally linear, although not necessarily over the full range (equivalent to 0.1-50 ng/g). In some cases (nortropinine, pseudotropine and scopoline) the s:n of the lower calibration standards was poor, therefore they were excluded from the graph. The lowest calibration standard was also excluded from several other graphs because the response at that concentration was non-linear. Some analytes showed non-linearity for the calibration standard equivalent to 50 ng/g; in most cases this can be attributed to saturation of the detector but for other analytes it is unexplained.

There were numerous interferences in the chromatograms, particularly for the lower m/z tropanes. In some cases (tropine, scopoline and convolvine) these interferences, or a high background, affected the analyte peak such that it was necessary to quantify using a different transition to that specified in the SOP (Annex 2 section 8.2.3). In cases where interferences could not be avoided and affect quantification of the spikes, the spike concentrations have been corrected by subtraction of the calculated concentration in the associated blank. In the case of nortropinone, an interference made it impossible to quantify the spikes at 1 ng/g.

Carry-over into the next injection(s) was observed for many of the analytes. Wash injections were included in the run, including washing with an injection of blank matrix because this seemed to be more effective than a solvent blank injection for some analytes. Carry-over appears as a very small residue in the matrix blanks for some injections, but not at a level that will affect quantification of the spikes.

Results for the analysis of each analyte in the six matrices included in the validation are shown in Table 2. If there was an interference or residue in the blank the results have been corrected by subtraction of this residue before reporting the mean or recovery values. No results are reported for the black tea QC samples because an LC pump error resulted in elution at the wrong retention time therefore peaks were lost outside the acquisition windows.

Because the validation spikes were quantified using calibration standards prepared in solvent, the value reported as “recovery” is actually a combination of recovery and the relative response of the analyte in matrix compared with solvent (i.e. matrix suppression or enhancement of the signal). The actual recovery of the extraction procedure can be measured by comparison of the MMRS samples, which were spiked after extraction and clean-up, with samples spiked before extraction and clean-up. The MMRS recovery results reported in Table 2 are calculated by comparison of the MMRS with the 5 ng/g and 25 ng/g spikes.

Results for atropine and scopolamine are inherently corrected for recovery and matrix effects by the inclusion of the internal standard. Calibration graphs for these compounds were prepared by plotting the ratio of the analyte : internal standard against concentration, rather than the peak area against concentration.

The large variation in “recovery” values for the same analyte in different types of tea highlights that it would not be possible to accurately quantify residues in a particular tea using matrix extracted standards in a different type of tea. For this reason, samples will be analysed using solvent calibration standards, with each sample analysed alongside an overspike to allow individual recovery values to be calculated.

QC samples were also run with each batch of tea samples. These QC consisted of blank green tea, a sample of this green tea spiked at 10 ng/g at Fera, a sample spiked at 10 ng/g at Rikilt and a blank extract spiked at a level equivalent to 10 ng/g after extraction and clean-up (i.e. MMRS). In addition, an MMRS was also prepared for each of the eight different types of tea which were sampled. These were used to calculate the actual recovery by comparison with the same sample spiked before extraction and clean-up. This actual recovery takes into account the recovery of the analyte through the whole analytical procedure and accounts for any matrix suppression or enhancement effects that occur in the mass spectrometer. Therefore it is a real indication of the performance of the method. Lemon balm gave the lowest recoveries of all the products tested in the validation with recoveries in the range 23 - 74%. Most alkaloids gave acceptable recovery performance, however the low molecular weight tropane alkaloid compounds tended to have lower recovery for all sample types. Repeatability was excellent for all analytes at all levels in all matrices tested with RSD values ranging from <1% to 19% for 6 replicate analyses at any individual level, even where recovery values were low.

The QC data for the tea batches is shown in Table 3. MMRS data is in table 4. Note that because the MMRS were prepared using real samples rather than blanks, the actual concentrations measured in the spikes and MMRS depends on whether there was any residue in the sample.

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Table 2: Validation results for tropane alkaloids in tea (low molecular weight).

Sample Type	Description	Nortropinone	Tropinone	Tropine	Pseudotropine	6-hydroxytropinone	Scopine	Scopoline
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Green tea	Blank	0.59 ^	0.00	0.00	(0.19) ^	0.00	0.94 ^	0.00
Green tea	Spike 1 1ng/g	0.39	0.53	0.98	1.19	0.22	1.44 ^	0.72
Green tea	Spike 2 1ng/g	0.35	0.56	0.95	1.18	0.22	1.50 ^	0.73
Green tea	Spike 3 1ng/g	0.41	0.51	1.03	1.13	0.22	1.48 ^	0.72
Green tea	Spike 4 1ng/g	0.39	0.53	1.01	1.20	0.24	1.50 ^	0.74
Green tea	Spike 5 1ng/g	0.43	0.52	1.02	1.24	0.22	1.34 ^	0.73
Green tea	Spike 6 1ng/g	0.34	0.47	0.93	1.23	0.25	1.42 ^	0.61
	Mean	**No data**	**0.52**	**0.99**	**1.00**	**0.23**	**0.51**	**0.71**
	"Recovery"	**No data**	**52%**	**99%**	**100%**	**23%**	**51%**	**71%**
	RSD	**9.4%**	**5.7%**	**4.1%**	**3.2%**	**5.0%**	**4.3%**	**6.6%**
Green tea	Spike 7 5ng/g	1.41	2.24	4.95	5.38	0.91	4.47	3.88
Green tea	Spike 8 5ng/g	1.46	2.27	5.06	5.27	0.91	4.48	3.67
Green tea	Spike 9 5ng/g	1.20	2.20	4.98	5.32	0.96	4.44	3.68
Green tea	Spike 10 5ng/g	1.55	2.24	5.09	5.22	0.97	4.52	3.74
Green tea	Spike 11 5ng/g	1.61	2.51	4.89	5.48	1.43	4.30	3.90
Green tea	Spike 12 5ng/g	1.46	2.60	5.06	5.09	1.00	4.77	3.90
	Mean	**0.86**	**2.34**	**5.01**	**5.10**	**1.03**	**3.56**	**3.79**
	"Recovery"	**17%**	**47%**	**100%**	**102%**	**21%**	**71%**	**76%**
	RSD	**9.8%**	**7.1%**	**1.6%**	**2.5%**	**19.1%**	**3.4%**	**2.9%**
Green tea	Spike 13 25ng/g	6.37	11.95	24.45	25.00	4.75	21.10	20.57
Green tea	Spike 14 25ng/g	7.35	13.77	24.76	25.34	4.96	21.18	20.44
Green tea	Spike 15 25ng/g	6.89	13.04	24.53	25.31	4.75	20.34	20.20
Green tea	Spike 16 25ng/g	6.32	12.45	24.53	25.73	4.92	20.63	20.33
Green tea	Spike 17 25ng/g	6.88	12.33	25.00	25.90	4.92	20.84	19.99
Green tea	Spike 18 25ng/g	6.58	12.88	24.65	25.55	5.15	20.83	19.47
	Mean	**6.14**	**12.74**	**24.65**	**25.28**	**4.91**	**19.88**	**20.17**
	"Recovery"	**25%**	**51%**	**99%**	**101%**	**20%**	**80%**	**81%**
	RSD	**5.8%**	**5.0%**	**0.8%**	**1.3%**	**3.0%**	**1.5%**	**2.0%**
Green tea	MMRS 1	6.44	12.54	11.81	12.98	4.26	10.99	10.03
Green tea	MMRS 2	6.28	12.99	12.37	12.94	4.38	11.24	10.90
	Mean	**5.77**	**12.76**	**12.09**	**12.76**	**4.32**	**10.17**	**10.46**
	Recovery	**36%**	**38%**	**82%**	**80%**	**46%**	**74%**	**75%**
Green tea	RIKILT QC Blank	0.55 ^	0.00	0.00	(0.22) ^	0.00	0.00	0.00
Green tea	RIKILT QC Spike	3.12	3.86	5.63	6.89	1.05	4.55	5.28
	"Recovery"	**26%**	**39%**	**56%**	**67%**	**10%**	**46%**	**53%**
Peppermint tea	S16-002253 QC Blank 1	(0.09) ^	0.00	0.00	(0.21) ^	0.00	0.00	0.00
Peppermint tea	S16-002253 QC Spike 1	3.44	1.29	6.69	4.41	2.78	3.05	1.53
	"Recovery"	**33%**	**13%**	**67%**	**42%**	**28%**	**31%**	**15%**
Fennel tea	S16-002254 QC Blank 2	(0.22) ^	(0.04) ^	0.85	(0.44) ^	0.00	0.00	0.00
Fennel tea	S16-002254 QC Spike 2	6.47	9.23	12.05	8.15	6.07	6.72	7.94
	"Recovery"	**62%**	**92%**	**112%**	**77%**	**61%**	**67%**	**79%**

^ = Presence of analyte not confirmed by ion ratio

( ) = Concentration reported is indicative only, due to being outside the calibration range, or due to the calibration graph failing acceptance criteria.

Table 2 (continued): Validation results for tropane alkaloids in tea (convolamine-type).

Sample Type	Description	3a-Phenylacetoxytropane	Convolidine	Fillalbin	Convolvine	Convolamine
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Green tea	Blank	(0.03)	0.00	0.00	(0.12)	(0.06)
Green tea	Spike 1 1ng/g	0.59	0.51	0.43	0.52	0.34
Green tea	Spike 2 1ng/g	0.59	0.54	0.42	0.53	0.31
Green tea	Spike 3 1ng/g	0.60	0.51	0.43	0.51	0.34
Green tea	Spike 4 1ng/g	0.60	0.51	0.44	0.51	0.34
Green tea	Spike 5 1ng/g	0.61	0.54	0.45	0.51	0.34
Green tea	Spike 6 1ng/g	0.59	0.52	0.44	0.50	0.33
	Mean	**0.60**	**0.52**	**0.44**	**0.51**	**0.33**
	"Recovery"	**60%**	**52%**	**44%**	**51%**	**33%**
	RSD	**1.5%**	**2.7%**	**1.8%**	**1.5%**	**3.1%**
Green tea	Spike 7 5ng/g	3.32	2.65	2.34	2.55	1.81
Green tea	Spike 8 5ng/g	3.31	2.64	2.34	2.57	1.80
Green tea	Spike 9 5ng/g	3.22	2.48	2.27	2.35	1.64
Green tea	Spike 10 5ng/g	3.10	2.53	2.15	2.38	1.63
Green tea	Spike 11 5ng/g	3.61	2.86	2.52	2.62	1.98
Green tea	Spike 12 5ng/g	3.38	2.58	2.39	2.49	1.80
	Mean	**3.32**	**2.62**	**2.34**	**2.49**	**1.78**
	"Recovery"	**66%**	**52%**	**47%**	**50%**	**36%**
	RSD	**5.1%**	**5.2%**	**5.3%**	**4.3%**	**7.2%**
Green tea	Spike 13 25ng/g	15.09	12.34	10.64	11.60	7.90
Green tea	Spike 14 25ng/g	17.09	13.19	12.01	12.27	9.02
Green tea	Spike 15 25ng/g	15.27	12.04	10.91	11.10	7.86
Green tea	Spike 16 25ng/g	15.93	12.92	10.93	11.56	8.13
Green tea	Spike 17 25ng/g	15.58	12.14	10.57	11.20	7.84
Green tea	Spike 18 25ng/g	16.73	12.85	11.35	12.09	8.78
	Mean	**15.95**	**12.58**	**11.07**	**11.64**	**8.25**
	"Recovery"	**64%**	**50%**	**44%**	**47%**	**33%**
	RSD	**5.0%**	**3.7%**	**4.9%**	**4.0%**	**6.3%**
Green tea	MMRS 1	12.20	8.69	9.37	11.95	10.63
Green tea	MMRS 2	12.49	9.12	9.45	11.91	10.80
	Mean	**12.35**	**8.90**	**9.41**	**11.93**	**10.72**
	Recovery	**53%**	**58%**	**48%**	**40%**	**32%**
Green tea	RIKILT QC Blank	(0.01) ^	0.00	0.00	0.00	(0.03)
Green tea	RIKILT QC Spike	6.06	4.07	4.24	5.01	4.50
	"Recovery"	**61%**	**41%**	**42%**	**50%**	**45%**
Peppermint tea	S16-002253 QC Blank 1	(0.02)	0.00	0.00	0.00	(0.05) ^
Peppermint tea	S16-002253 QC Spike 1	10.69	7.21	8.47	11.06	12.39
	"Recovery"	**107%**	**72%**	**85%**	**111%**	**124%**
Fennel tea	S16-002254 QC Blank 2	(0.02) ^	0.00	0.11	0.00	(0.04) ^
Fennel tea	S16-002254 QC Spike 2	9.22	7.64	7.66	8.68	6.13
	"Recovery"	**92%**	**76%**	**76%**	**87%**	**61%**

^ = Presence of analyte not confirmed by ion ratio

( ) = Concentration reported is indicative only, due to being outside the calibration range, or due to the calibration graph failing acceptance criteria.

Annex 4. Fera Validation report for tropane alkaloids in teas

Table 2 (continued): Validation results for tropane alkaloids in tea (atropine-type part 1).

Sample Type	Description	Apoatropine	Noratropine	Homatropine	Aposcopolamine	Norscopolamine	Atropine	Atropine D3
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Rel Resp
Green tea	Blank	(0.09)	(0.02)	(0.01)	0.00	0.00	(0.03)	74%
Green tea	Spike 1 1ng/g	0.55	0.79	0.79	0.60	0.56	1.01	69%
Green tea	Spike 2 1ng/g	0.52	0.80	0.75	0.61	0.58	1.03	68%
Green tea	Spike 3 1ng/g	0.55	0.80	0.79	0.61	0.58	1.06	67%
Green tea	Spike 4 1ng/g	0.57	0.80	0.77	0.61	0.61	1.09	65%
Green tea	Spike 5 1ng/g	0.55	0.77	0.78	0.60	0.59	1.08	65%
Green tea	Spike 6 1ng/g	0.54	0.76	0.76	0.60	0.60	1.13	62%
	Mean	**0.55**	**0.79**	**0.77**	**0.61**	**0.59**	**1.07**	**66%**
	"Recovery"	**55%**	**79%**	**77%**	**61%**	**59%**	**107%**	**66%**
	RSD	**3.1%**	**2.4%**	**1.9%**	**1.2%**	**3.2%**	**3.8%**	**3.6%**
Green tea	Spike 7 5ng/g	2.88	3.98	4.14	3.00	3.19	5.81	64%
Green tea	Spike 8 5ng/g	2.81	3.97	4.09	3.23	3.17	5.22	71%
Green tea	Spike 9 5ng/g	2.71	4.07	4.06	3.12	3.28	5.81	62%
Green tea	Spike 10 5ng/g	2.62	3.90	3.81	2.98	3.22	5.26	65%
Green tea	Spike 11 5ng/g	3.13	4.11	4.49	3.62	3.23	5.42	75%
Green tea	Spike 12 5ng/g	2.92	4.00	4.16	3.30	3.25	5.36	70%
	Mean	**2.84**	**4.00**	**4.12**	**3.21**	**3.22**	**5.48**	**68%**
	"Recovery"	**57%**	**80%**	**82%**	**64%**	**64%**	**110%**	**68%**
	RSD	**6.3%**	**1.9%**	**5.3%**	**7.5%**	**1.2%**	**4.8%**	**7.2%**
Green tea	Spike 13 25ng/g	13.14	18.35	19.05	15.18	14.71	27.38	62%
Green tea	Spike 14 25ng/g	14.67	20.06	22.08	16.18	15.65	25.78	74%
Green tea	Spike 15 25ng/g	12.88	18.63	20.12	14.88	15.40	29.32	61%
Green tea	Spike 16 25ng/g	13.29	18.74	20.17	15.37	15.39	23.95	72%
Green tea	Spike 17 25ng/g	13.44	18.84	20.31	15.17	15.43	25.22	71%
Green tea	Spike 18 25ng/g	14.26	19.52	21.36	16.14	15.78	25.31	73%
	Mean	**13.61**	**19.02**	**20.51**	**15.49**	**15.39**	**26.16**	**69%**
	"Recovery"	**54%**	**76%**	**82%**	**62%**	**62%**	**105%**	**69%**
	RSD	**5.1%**	**3.4%**	**5.2%**	**3.5%**	**2.4%**	**7.3%**	**8.6%**
Green tea	MMRS 1	14.36	10.27	11.82	10.63	7.11	14.36	76%
Green tea	MMRS 2	14.66	10.43	11.86	10.94	7.24	14.56	77%
	Mean	**14.51**	**10.35**	**11.84**	**10.79**	**7.18**	**14.46**	**0.76**
	Recovery	**38%**	**75%**	**69%**	**58%**	**88%**	**74%**	**89%**
Green tea	RIKILT QC Blank	(0.05) ^	0.00	0.00	0.00	(0.04) ^	(0.02)	80%
Green tea	RIKILT QC Spike	5.58	4.88	6.03	4.95	3.77	6.91	81%
	"Recovery"	**56%**	**49%**	**60%**	**50%**	**37%**	**69%**	**81%**
Peppermint tea	S16-002253 QC Blank 1	(0.07) ^	0.00	0.00	0.00	0.00	(0.04)	100%
Peppermint tea	S16-002253 QC Spike 1	11.13	8.71	10.14	8.78	6.97	9.94	99%
	"Recovery"	**111%**	**87%**	**101%**	**88%**	**70%**	**99%**	**99%**
Fennel tea	S16-002254 QC Blank 2	(0.06)	(0.03) ^	0.00	0.00	(0.08) ^	(0.04)	86%
Fennel tea	S16-002254 QC Spike 2	8.10	9.88	10.61	9.08	8.87	9.76	98%
	"Recovery"	**81%**	**99%**	**106%**	**91%**	**88%**	**98%**	**98%**

^ = Presence of analyte not confirmed by ion ratio

( ) = Concentration reported is indicative only, due to being outside the calibration range, or due to the calibration graph failing acceptance criteria.

Annex 4. Fera Validation report for tropane alkaloids in teas

Table 2 (continued): Validation results for tropane alkaloids in tea (atropine-type part 2).

Sample Type	Description	Littorine	Scopolamine	Scopolamine D3	Anisodamine	Anisodine	2a-OH-methylatropine	o-Acetylscopolamine
		Conc / ng/g	Conc / ng/g	Rel Resp	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Green tea	Blank	(0.02)	0.00	77%	0.00	0.00	0.00	0.00
Green tea	Spike 1 1ng/g	0.75	0.88	73%	0.79	0.66	0.80	0.50
Green tea	Spike 2 1ng/g	0.75	0.89	74%	0.78	0.65	0.79	0.43
Green tea	Spike 3 1ng/g	0.74	0.88	74%	0.79	0.66	0.78	0.37
Green tea	Spike 4 1ng/g	0.74	0.90	71%	0.79	0.69	0.78	0.50
Green tea	Spike 5 1ng/g	0.74	0.91	70%	0.79	0.65	0.78	0.44
Green tea	Spike 6 1ng/g	0.74	0.97	66%	0.79	0.64	0.80	0.33
	Mean	**0.74**	**0.90**	**71%**	**0.79**	**0.66**	**0.79**	**0.43**
	"Recovery"	**74%**	**90%**	**71%**	**79%**	**66%**	**79%**	**43%**
	RSD	**0.7%**	**3.8%**	**4.4%**	**0.5%**	**2.7%**	**1.0%**	**16%**
Green tea	Spike 7 5ng/g	3.99	5.00	66%	4.16	3.63	4.08	2.55
Green tea	Spike 8 5ng/g	3.93	4.49	74%	4.05	3.62	4.06	2.45
Green tea	Spike 9 5ng/g	3.91	5.16	65%	4.16	3.65	4.12	2.30
Green tea	Spike 10 5ng/g	3.69	4.45	73%	4.01	3.66	3.92	2.19
Green tea	Spike 11 5ng/g	4.26	4.62	74%	4.20	3.70	4.29	2.55
Green tea	Spike 12 5ng/g	4.03	4.61	72%	4.13	3.68	4.17	3.03
	Mean	**3.97**	**4.72**	**71%**	**4.12**	**3.66**	**4.11**	**2.51**
	"Recovery"	**79%**	**94%**	**71%**	**82%**	**73%**	**82%**	**50%**
	RSD	**4.7%**	**6.2%**	**5.7%**	**1.8%**	**0.8%**	**3.0%**	**12%**
Green tea	Spike 13 25ng/g	18.99	22.75	66%	19.91	17.09	18.81	12.47
Green tea	Spike 14 25ng/g	21.59	22.03	74%	21.13	18.50	21.07	15.84
Green tea	Spike 15 25ng/g	19.56	23.13	65%	20.28	17.35	19.24	14.13
Green tea	Spike 16 25ng/g	19.64	20.32	75%	19.63	17.54	19.32	13.84
Green tea	Spike 17 25ng/g	19.37	20.69	74%	20.74	17.76	19.03	12.49
Green tea	Spike 18 25ng/g	20.44	20.81	78%	20.09	17.70	19.78	14.05
	Mean	**19.93**	**21.62**	**72%**	**20.30**	**17.66**	**19.54**	**13.80**
	"Recovery"	**80%**	**86%**	**72%**	**81%**	**71%**	**78%**	**55%**
	RSD	**4.7%**	**5.5%**	**7.2%**	**2.7%**	**2.7%**	**4.2%**	**9.1%**
Green tea	MMRS 1	11.62	8.75	80%	9.67	8.33	10.30	25.86
Green tea	MMRS 2	11.96	9.03	81%	10.12	8.70	10.77	26.58
	Mean	**11.79**	**8.89**	**0.80**	**9.90**	**8.51**	**10.54**	**26.22**
	Recovery	**67%**	**102%**	**89%**	**83%**	**84%**	**76%**	**20%**
Green tea	RIKILT QC Blank	0.00	0.00	81%	0.00	0.00	0.00	0.00
Green tea	RIKILT QC Spike	5.91	5.33	76%	4.88	4.39	5.33	8.71
	"Recovery"	**59%**	**53%**	**76%**	**49%**	**44%**	**53%**	**87%**
Peppermint tea	S16-002253 QC Blank 1	0.00	(0.02)	60%	0.00	0.00	0.00	0.00
Peppermint tea	S16-002253 QC Spike 1	10.64	8.44	62%	7.34	4.73	6.78	4.84
	"Recovery"	**106%**	**84%**	**62%**	**73%**	**47%**	**68%**	**48%**
Fennel tea	S16-002254 QC Blank 2	0.00	(0.03)	84%	0.00	0.00	0.00	0.00
Fennel tea	S16-002254 QC Spike 2	10.57	7.85	92%	9.60	8.93	9.66	8.64
	"Recovery"	**106%**	**78%**	**92%**	**96%**	**89%**	**97%**	**86%**

^ = Presence of analyte not confirmed by ion ratio

( ) = Concentration reported is indicative only, due to being outside the calibration range, or due to the calibration graph failing acceptance criteria.

Annex 4. Fera Validation report for tropane alkaloids in teas

Table 3: Batch QC results for tropane alkaloids in tea (low molecular weight).

Sample Type	Description	Nortropinone	Tropinone	Tropine	Pseudotropine	6-hydroxytropinone	Scopine	Scopoline
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Green tea	Batch 1 QC Blank	0.25	0.00	0.00	0.00	0.00	0.26	0.00
Green tea	Batch 1 QC Spike 10 ng/g	3.69	4.77	6.81	8.39	2.91	7.12	6.24
Green tea	Batch 1 Rikilt Spike 10 ng/g	4.10	5.80	8.29	9.56	3.39	7.54	7.64
Green tea	Batch 1 MMRS	5.58	9.67	9.24	10.46	4.20	8.86	8.70
	Batch 1 Rikilt "Recovery"	**38%**	**58%**	**83%**	**96%**	**34%**	**73%**	**76%**
	Batch 1 Fera "Recovery"	**34%**	**48%**	**68%**	**84%**	**29%**	**69%**	**62%**
	Batch 1 Actual Recovery	**66%**	**49%**	**74%**	**80%**	**69%**	**80%**	**72%**
Green tea	Batch 2 QC Blank	0.23	0.00	0.00	0.00	0.00	0.00	0.00
Green tea	Batch 2 QC Spike 10 ng/g	3.78	(3.93)	(5.59)	8.01	2.67	(6.44)	(6.17)
Green tea	Batch 2 Rikilt Spike 10 ng/g	4.65	(5.37)	(7.26)	11.65	3.33	(8.24)	(7.88)
Green tea	Batch 2 MMRS	7.66	(12.20)	(8.40)	12.58	4.66	(9.79)	(11.29)
	Batch 2 Rikilt "Recovery"	**44%**	**54%**	**73%**	**117%**	**33%**	**82%**	**79%**
	Batch 2 Fera "Recovery"	**36%**	**39%**	**56%**	**80%**	**27%**	**64%**	**62%**
	Batch 2 Actual Recovery	**49%**	**32%**	**67%**	**64%**	**57%**	**66%**	**55%**

^ = Presence of analyte not confirmed by ion ratio
( ) = Concentration reported is indicative only, due to being outside the calibration range, or due to the calibration graph failing acceptance criteria.

Table 3 (continued): Batch QC results for tropane alkaloids in tea (convolamine-type).

Sample Type	Description	3a-Phenylacetoxytropane	Convolidine	Fillalbin	Convolvine	Convolamine
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Green tea	Batch 1 QC Blank	(0.18)	(0.11)	0.00	(0.24)	(0.21)
Green tea	Batch 1 QC Spike 10 ng/g	(16.64)	(4.40) ^	(8.51)	(16.62)	(17.19)
Green tea	Batch 1 Rikilt Spike 10 ng/g	(19.35)	(5.25) ^	(9.90)	(19.38)	(20.21)
Green tea	Batch 1 MMRS	(23.50)	(6.76) ^	(11.35)	(26.46)	(24.91)
	Batch 1 Rikilt "Recovery"	**192%**	**51%**	**99%**	**191%**	**200%**
	Batch 1 Fera "Recovery"	**165%**	**43%**	**85%**	**164%**	**170%**
	Batch 1 Actual Recovery	**71%**	**65%**	**75%**	**63%**	**69%**
Green tea	Batch 2 QC Blank	(0.15)	0.00	0.00	(0.19)	(0.21)
Green tea	Batch 2 QC Spike 10 ng/g	(9.64)	(4.01)	(6.41)	(8.28)	(7.81)
Green tea	Batch 2 Rikilt Spike 10 ng/g	(13.45)	5.04 ^	(9.78)	(11.58)	(11.36)
Green tea	Batch 2 MMRS	(14.59)	6.45 ^	(10.07)	(14.31)	(12.70)
	Batch 2 Rikilt "Recovery"	**133%**	**50%**	**98%**	**114%**	**112%**
	Batch 2 Fera "Recovery"	**95%**	**40%**	**64%**	**81%**	**76%**
	Batch 2 Actual Recovery	**66%**	**62%**	**64%**	**58%**	**61%**

^ = Presence of analyte not confirmed by ion ratio
( ) = Concentration reported is indicative only, due to being outside the calibration range, or due to the calibration graph failing acceptance criteria.

Table 3 (continued): Batch QC results for tropane alkaloids in tea (atropine-type part 1).

Sample Type	Description	Apoatropine	Noratropine	Homatropine	Aposcopolamine	Norscopolamine	Atropine	Atropine D3
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Rel Resp
Green tea	Batch 1 QC Blank	(0.26)	(0.08)	(0.12)	0.05	0.00	0.03	107%
Green tea	Batch 1 QC Spike 10 ng/g	(25.35)	(6.01)	(9.81)	9.63	5.69	12.31	100%
Green tea	Batch 1 Rikilt Spike 10 ng/g	(30.14)	(7.08)	(11.05)	11.11	6.61	12.71	109%
Green tea	Batch 1 MMRS	(41.80)	(8.15)	(12.78)	14.25	7.09	14.03	113%
	Batch 1 Rikilt "Recovery"	**299%**	**70%**	**109%**	**111%**	**66%**	**127%**	**109%**
	Batch 1 Fera "Recovery"	**251%**	**59%**	**97%**	**96%**	**57%**	**123%**	**100%**
	Batch 1 Actual Recovery	**61%**	**74%**	**77%**	**68%**	**80%**	**88%**	**88%**
Green tea	Batch 2 QC Blank	(0.21)	0.03	0.07	0.05	0.00	0.03	78%
Green tea	Batch 2 QC Spike 10 ng/g	(11.87)	6.63	8.96	6.97	5.42	11.37	78%
Green tea	Batch 2 Rikilt Spike 10 ng/g	(16.17)	9.18	11.85	9.26	7.39	15.44	80%
Green tea	Batch 2 MMRS	(20.00)	10.51	12.54	11.63	7.85	14.78	87%
	Batch 2 Rikilt "Recovery"	**160%**	**92%**	**118%**	**92%**	**74%**	**154%**	**80%**
	Batch 2 Fera "Recovery"	**117%**	**66%**	**89%**	**69%**	**54%**	**113%**	**78%**
	Batch 2 Actual Recovery	**59%**	**63%**	**71%**	**60%**	**69%**	**77%**	**90%**

^ = Presence of analyte not confirmed by ion ratio
( ) = Concentration reported is indicative only, due to being outside the calibration range, or due to the calibration graph failing acceptance criteria.

Annex 4. Fera Validation report for tropane alkaloids in teas

Table 3 (continued): Batch QC results for tropane alkaloids in tea (atropine-type part 2).

Sample Type	Description	Littorine	Scopolamine	Scopolamine D3	Anisodamine	Anisodine	2a-OH-methylatropine	o-Acetylscopolamine
		Conc / ng/g	Conc / ng/g	Rel Resp	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Green tea	Batch 1 QC Blank	(0.13)	0.00	74%	0.03	0.00	0.00	0.00
Green tea	Batch 1 QC Spike 10 ng/g	(11.07)	11.14	70%	7.36	6.51	6.57	9.03
Green tea	Batch 1 Rikilt Spike 10 ng/g	(12.79)	12.46	74%	8.60	7.71	7.95	10.95
Green tea	Batch 1 MMRS	(14.39)	12.33	79%	9.32	8.46	8.93	13.59
	Batch 1 Rikilt "Recovery"	**127%**	**125%**	**74%**	**86%**	**77%**	**80%**	**109%**
	Batch 1 Fera "Recovery"	**109%**	**111%**	**70%**	**73%**	**65%**	**66%**	**90%**
	Batch 1 Actual Recovery	**77%**	**90%**	**89%**	**79%**	**77%**	**74%**	**66%**
Green tea	Batch 2 QC Blank	(0.11)	0.00	73%	0.01	0.00	0.00	0.00
Green tea	Batch 2 QC Spike 10 ng/g	(8.79)	10.02	72%	6.59	6.43	4.94	8.17
Green tea	Batch 2 Rikilt Spike 10 ng/g	(11.53)	13.31	71%	8.79	8.78	7.14	10.71
Green tea	Batch 2 MMRS	(12.35)	12.77	76%	9.42	9.24	7.27	12.85
	Batch 2 Rikilt "Recovery"	**114%**	**133%**	**71%**	**88%**	**88%**	**71%**	**107%**
	Batch 2 Fera "Recovery"	**87%**	**100%**	**72%**	**66%**	**64%**	**49%**	**82%**
	Batch 2 Actual Recovery	**71%**	**78%**	**96%**	**70%**	**70%**	**68%**	**64%**

^ = Presence of analyte not confirmed by ion ratio
( ) = Concentration reported is indicative only, due to being outside the calibration range, or due to the calibration graph failing acceptance criteria.

Annex 4. Fera Validation report for tropane alkaloids in teas

Table 4: MMRS results for tropane alkaloids in tea (low molecular weight).

Sample Type	Description	Nortropinone	Tropinone	Tropine	Pseudotropine	6-hydroxytropinone	Scopine	Scopoline
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Chamomile tea	S16-003231 Spike	3.06	1.16	(54.15) ^	7.14	3.37	3.98	3.29
Chamomile tea	S16-003231 MMRS	5.04	3.11	(52.44) ^	11.69	5.05	8.04	6.86
Chamomile Tea Actual Recovery		**61%**	**37%**	**103%**	**61%**	**67%**	**49%**	**48%**
Peppermint tea	S16-003156 Spike	4.20	1.31	10.57	9.48	3.51	3.58	3.47
Peppermint tea	S16-003156 MMRS	9.69	6.93	15.97	18.80	6.12	10.56	10.54
Peppermint Tea Actual Recovery		**43%**	**19%**	**66%**	**50%**	**57%**	**34%**	**33%**
Green tea	S16-003235 Spike	3.98	3.26	7.18	9.58	3.16	6.79	6.66
Green tea	S16-003235 MMRS	7.87	9.90	10.42	13.38	5.53	9.48	9.80
	Green Tea Actual Recovery	**51%**	**33%**	**69%**	**72%**	**57%**	**72%**	**68%**
Lemon Balm tea	S16-003252 Spike	3.99	2.29	7.76	9.23	3.16	4.13	4.07
Lemon Balm tea	S16-003252 MMRS	10.31	10.17	14.47	16.44	7.21	11.02	10.52
Lemon Balm Tea Actual Recovery		**39%**	**23%**	**54%**	**56%**	**44%**	**37%**	**39%**
Black tea	S16-003236 Spike	3.64	(4.08)	(6.70)	7.65	2.43	(5.94)	(6.88)
Black tea	S16-003236 MMRS	7.77	(9.64)	(9.30)	12.16	5.01	(8.63)	(10.18)
	Black Tea Actual Recovery	**47%**	**42%**	**72%**	**63%**	**49%**	**69%**	**68%**
Redbush tea	S16-003155 Spike	4.41	(4.77)	(7.10)	9.63	3.00	(6.01)	(7.05)
Redbush tea	S16-003155 MMRS	7.40	(9.18)	(9.71)	11.83	5.18	(8.19)	(8.83)
	Redbush Tea Actual Recovery	**60%**	**52%**	**73%**	**81%**	**58%**	**73%**	**80%**
Nettle tea	S16-003154 Spike	1.28	(1.07)	(4.47)	4.16	1.96	(1.79)	(1.77)
Nettle tea	S16-003154 MMRS	5.42	(10.70)	(11.51)	15.58	6.29	(9.61)	(9.58)
	Nettle Tea Actual Recovery	**24%**	**10%**	**39%**	**27%**	**31%**	**19%**	**18%**
Mint tea	S16-003310 Spike	3.30	(1.43)	(7.17)	7.70	3.46	(4.56)	(4.66)
Mint tea	S16-003310 MMRS	8.39	(7.74)	(12.19)	14.92	5.72	(8.87)	(10.85)
	Mint Tea Actual Recovery	**39%**	**18%**	**59%**	**52%**	**60%**	**51%**	**43%**

^ = Presence of analyte not confirmed by ion ratio

( ) = Concentration reported is indicative only, due to being outside the calibration range, or due to the calibration graph failing acceptance criteria.

Annex 4. Fera Validation report for tropane alkaloids in teas

Table 4 (continued): MMRS results for tropane alkaloids in tea (convolamine-type).

Sample Type	Description	3a-Phenylacetoxytropane	Convolidine	Fillalbin	Convolvine	Convolamine
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Chamomile tea	S16-003231 Spike	(17.80)	(5.93) ^	(11.60)	(17.72)	(21.77)
Chamomile tea	S16-003231 MMRS	(23.07)	(6.17) ^	(11.91)	(25.39)	(28.37)
Chamomile Tea Actual Recovery		**77%**	**96%**	**97%**	**70%**	**77%**
Peppermint tea	S16-003156 Spike	(13.82)	(4.73)	(6.29)	(13.29)	(13.21)
Peppermint tea	S16-003156 MMRS	(22.40)	(7.82)	(11.32)	(24.27)	(23.70)
Peppermint Tea Actual Recovery		**62%**	**60%**	**56%**	**55%**	**56%**
Green tea	S16-003235 Spike	(13.91)	(4.05) ^	(6.42)	(12.40)	(11.09)
Green tea	S16-003235 MMRS	(16.96)	(6.11)	(8.12)	(17.91)	(12.72)
	Green Tea Actual Recovery	**82%**	**66%**	**79%**	**69%**	**87%**
Lemon Balm tea	S16-003252 Spike	(8.45)	(3.43) ^	(5.11)	(10.00)	(11.63)
Lemon Balm tea	S16-003252 MMRS	(22.65)	(8.37) ^	(10.76)	(23.09)	(25.64)
Lemon Balm Tea Actual Recovery		**37%**	**41%**	**47%**	**43%**	**45%**
Black tea	S16-003236 Spike	(2.55)	(1.38)	(1.30)	(1.37)	(0.80)
Black tea	S16-003236 MMRS	(3.95)	(2.33) ^	(2.26)	(2.38)	(1.21)
	Black Tea Actual Recovery	**65%**	**59%**	**58%**	**57%**	**67%**
Redbush tea	S16-003155 Spike	(8.23)	(2.35)	(3.51)	(4.43)	(4.33)
Redbush tea	S16-003155 MMRS	(13.97)	(5.08) ^	(7.25)	(10.81)	(11.59)
Redbush Tea Actual Recovery		**59%**	**46%**	**48%**	**41%**	**37%**
Nettle tea	S16-003154 Spike	(13.35)	(5.10) ^	(9.50)	(13.21)	(17.09)
Nettle tea	S16-003154 MMRS	(18.74)	(7.38) ^	(12.66)	(18.03)	(21.41)
	Nettle Tea Actual Recovery	**71%**	**69%**	**75%**	**73%**	**80%**
Mint tea	S16-003310 Spike	(7.69)	(3.66)	(4.98)	(6.18)	(5.24)
Mint tea	S16-003310 MMRS	(11.89)	(6.15) ^	(7.26)	(10.44)	(7.96)
	Mint Tea Actual Recovery	**65%**	**59%**	**69%**	**59%**	**66%**

^ = Presence of analyte not confirmed by ion ratio

( ) = Concentration reported is indicative only, due to being outside the calibration range, or due to the calibration graph failing acceptance criteria.

Annex 4. Fera Validation report for tropane alkaloids in teas

Table 4 (continued): MMRS results for tropane alkaloids in tea (atropine-type part 1).

Sample Type	Description	Apoatropine	Noratropine	Homatropine	Aposcopolamine	Norscopolamine	Atropine	Atropine D3
		Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g	Rel Resp
Chamomile tea	S16-003231 Spike	(29.85)	(5.69)	(8.62)	9.64	4.59	10.50	104%
Chamomile tea	S16-003231 MMRS	(42.66)	(6.55)	(10.03)	12.93	5.15	15.99	82%
Chamomile Tea Actual Recovery		**70%**	**87%**	**86%**	**75%**	**89%**	**66%**	**126%**
Peppermint tea	S16-003156 Spike	(20.86)	(6.91)	(8.42)	8.17	5.33	13.57	83%
Peppermint tea	S16-003156 MMRS	(36.36)	(10.16)	(12.43)	13.99	7.79	17.75	93%
Peppermint Tea Actual Recovery		**57%**	**68%**	**68%**	**58%**	**68%**	**76%**	**89%**
Green tea	S16-003235 Spike	(19.29)	(7.39)	(9.11)	7.86	5.55	14.11	83%
Green tea	S16-003235 MMRS	(26.80)	(10.43)	(11.44)	10.72	7.34	15.11	90%
	Green Tea Actual Recovery	**72%**	**71%**	**80%**	**73%**	**76%**	**93%**	**93%**
Lemon Balm tea	S16-003252 Spike	(14.63)	(4.71)	(4.21)	3.94	2.37	10.84	62%
Lemon Balm tea	S16-003252 MMRS	(37.54)	(9.81)	(12.89)	13.84	7.70	19.86	78%
Lemon Balm Tea Actual Recovery		**39%**	**48%**	**33%**	**28%**	**31%**	**55%**	**80%**
Black tea	S16-003236 Spike	(2.04)	4.31	4.20	1.61	4.04	12.31	29%
Black tea	S16-003236 MMRS	(3.56)	6.25	5.95	3.13	5.46	18.87	27%
	Black Tea Actual Recovery	**57%**	**69%**	**71%**	**52%**	**74%**	**65%**	**106%**
Redbush tea	S16-003155 Spike	(8.69)	6.02	7.80	5.50	4.41	12.23	64%
Redbush tea	S16-003155 MMRS	(17.06)	8.37	10.76	10.01	5.65	16.79	67%
	Redbush Tea Actual Recovery	**51%**	**72%**	**72%**	**55%**	**78%**	**73%**	**96%**
Nettle tea	S16-003154 Spike	(17.43)	7.39	9.93	8.42	4.91	11.95	87%
Nettle tea	S16-003154 MMRS	(28.82)	9.26	12.25	13.79	6.78	15.06	87%
	Nettle Tea Actual Recovery	**61%**	**80%**	**81%**	**61%**	**72%**	**79%**	**99%**
Mint tea	S16-003310 Spike	(8.56)	6.82	7.61	5.51	5.01	11.52	72%
Mint tea	S16-003310 MMRS	(14.09)	10.00	10.59	9.53	6.96	14.53	76%
	Mint Tea Actual Recovery	**61%**	**68%**	**72%**	**58%**	**72%**	**79%**	**95%**

^ = Presence of analyte not confirmed by ion ratio
( ) = Concentration reported is indicative only, due to being outside the calibration range, or due to the calibration graph failing acceptance criteria.

Annex 4. Fera Validation report for tropane alkaloids in teas

Table 4 (continued): MMRS results for tropane alkaloids in tea (atropine-type part 2).

Sample Type	Description	Littorine	Scopolamine	Scopolamine D3	Anisodamine	Anisodine	2a-OH-methylatropine	o-Acetylscopolamine
		Conc / ng/g	Conc / ng/g	Rel Resp	Conc / ng/g	Conc / ng/g	Conc / ng/g	Conc / ng/g
Chamomile tea	S16-003231 Spike	(11.00)	10.33	60%	6.68	5.32	5.43	7.42
Chamomile tea	S16-003231 MMRS	(12.68)	14.94	50%	7.55	6.49	5.79	12.82
Chamomile Tea Actual Recovery		**87%**	**69%**	**121%**	**88%**	**82%**	**94%**	**58%**
Peppermint tea	S16-003156 Spike	(10.16)	12.15	50%	6.79	4.77	4.69	6.52
Peppermint tea	S16-003156 MMRS	(14.68)	19.07	53%	10.04	8.94	8.10	13.67
Peppermint Tea Actual Recovery		**69%**	**64%**	**94%**	**68%**	**53%**	**58%**	**48%**
Green tea	S16-003235 Spike	(10.05)	12.41	61%	7.17	6.36	6.09	8.15
Green tea	S16-003235 MMRS	(12.78)	12.72	68%	8.86	8.36	7.51	10.73
	Green Tea Actual Recovery	**79%**	**98%**	**89%**	**81%**	**76%**	**81%**	**76%**
Lemon Balm tea	S16-003252 Spike	(8.29)	9.84	23%	4.73	2.55	4.66	1.70
Lemon Balm tea	S16-003252 MMRS	(14.13)	(39.36)	26%	9.61	8.54	7.98	13.33
Lemon Balm Tea Actual Recovery		**59%**	**25%**	**88%**	**49%**	**30%**	**58%**	**13%**
Black tea	S16-003236 Spike	(3.63)	11.44	33%	4.55	4.49	2.95	2.34
Black tea	S16-003236 MMRS	(5.11)	15.89	32%	6.13	6.55	4.65	3.99
	Black Tea Actual Recovery	**71%**	**72%**	**102%**	**74%**	**69%**	**64%**	**59%**
Redbush tea	S16-003155 Spike	(7.70)	10.96	54%	5.77	5.37	4.40	6.66
Redbush tea	S16-003155 MMRS	(10.78)	14.69	56%	7.49	7.18	6.24	11.48
Redbush Tea Actual Recovery		**71%**	**75%**	**97%**	**77%**	**75%**	**70%**	**58%**
Nettle tea	S16-003154 Spike	(10.74)	10.93	48%	5.82	3.60	3.39	7.11
Nettle tea	S16-003154 MMRS	(12.57)	18.96	48%	8.77	8.50	6.42	14.24
	Nettle Tea Actual Recovery	**85%**	**58%**	**101%**	**66%**	**42%**	**53%**	**50%**
Mint tea	S16-003310 Spike	(8.21)	10.26	63%	6.44	5.72	4.40	6.71
Mint tea	S16-003310 MMRS	(10.84)	12.94	70%	8.80	8.16	6.01	11.03
	Mint Tea Actual Recovery	**76%**	**79%**	**91%**	**73%**	**70%**	**73%**	**61%**

^ = Presence of analyte not confirmed by ion ratio

( ) = Concentration reported is indicative only, due to being outside the calibration range, or due to the calibration graph failing acceptance criteria.

Annex 5. Method of analysis for calystegines in vegetables

Standard Operation Protocol for LC-MS/MS analysis of calystegines in potato and aubergine samples

SOP for EFSA project Occurrence of TAs in food

Patrick Mulder, February-April 2016, version 1a

Introduction

- This document describes the validated SOP for the LC-MS/MS analysis of calystegines in potato and aubergine samples in the range of 1 to 100 mg/kg.

TAs are extracted from the homogenised product with an acidic aqueous/acetonitrile solution. The extracts are diluted with ammonium acetate buffer and analysed by LC-MS/MS.

Part A: Standards, solutions and equipment

1. Mixed standard solutions of calystegines (1 and 10 µg/ml) in methanol

1.1 Calystegines included in the method are:

- Calystegine A_3, calystegine A_5, calystegine B_1, calystegine B_2, calystegine B_3, calystegine B_4.

These calystegines are available as stock solutions (100 µg/ml) in methanol.

1.2 Calystegines mixed standard solution (10 µg/ml) in methanol.

- Take 100 µl of each TA stock solution (100 µg/ml), add 400 µl methanol and mix.

1.3 Calystegines mixed standard spiking solution (1 µg/ml) in methanol.

- Take 1.0 ml of mixed standard solution (10 µg/ml), add 9.0 ml of methanol and mix.

All solutions are stored at -20ºC in the dark for the duration of the project.

2. Extraction solutions

2.1 Solvents and chemicals.

2.1.1 Formic acid, glacial.

2.1.2 Methanol, UPLC quality.

2.1.3 Acetonitrile, UPLC quality.

2.1.4 Ammonium acetate, anhydrous, p.a. quality.

2.1.5 Acetic acid, glacial

2.1.6 Ammonia, 25% in water

2.1.7 Water, purified by a Milli-Q purification system, having a minimal resistance of 18.2 MΩ/cm.

2.2 Solutions

2.2.1 Extraction solvent: acetonitrile/water/formic acid solution (50/50/0.2%).

- Mix 2 ml formic acid (2.1.1) with 500 ml acetonitrile (2.1.3) and 500 ml water (2.1.7).

The solution is stored at room temperature and can be used for 3 months.

2.2.2 Mobile phase A for LC-MS/MS: 5 mM ammonium acetate pH 7.0.

- Dissolve 0.41 g ammonium acetate (2.1.4) in 1 l water (2.1.7) and adjust to pH 7.0 with ammonia 25% (2.1.6) or acetic acid (2.1.5).

The solution is stored at room temperature and can be used for 1 week

2.2.3 Mobile phase B for LC-MS/MS: methanol (2.1.2).

2.2.4 Working standard solutions for quantification
Prepare WS for quantification according to the table:

WS (ng/ml)	Relates to potato matrix (mg/kg)	Mixed calystegines solution 1 µg/ml (1.3) (µl)	Mixed calystegines solution 10 µg/ml (1.2) (µl)	Mobile phase A (2.2.2) (µl)
0	0	-	-	1000
10	1	10	-	990
25	2.5	25	-	975
50	5	50	-	950
100	10	100	-	900
250	25	-	25	975
500	50	-	50	950
1000	100	-	100	900
2000	200	-	200	800

These solutions should be prepared every new day of analysis.

3. Equipment
3.1 Balance with a minimal weight range of 0 until 500 gram and with a precision of 0.01 g (e.g. Mettler Toledo GP503-S).
3.2 Vortex mixer (e.g. IKA Vortex 3).
3.3 Overhead shaker (e.g. Heidolph REAX 2).
3.4 Centrifuge suitable for 12 and 50 ml centrifuge tubes (e.g. MSE Falcon 6/300R).
3.5 Polypropylene tubes of 50 ml with screw cap (e.g. Greiner 210261).
3.6 Dispenser 5-50 ml (e.g. Dispensette 4701361).
3.7 Pipettes for various solvent volumes, including a positive displacement pipet for organic solvents (e.g. Pipetman).
3.8 Filtervial PTFE 0.45 µm, 500 µl (e.g. Whatman UN203NPUORG).
3.9 Compressor for filtervials 6 positions (e.g. Whatman CR0000006).
3.10 pH meter

Part B: Extraction and clean-up

4. Sample pre-treatment and test sample size for potatoes and aubergines

- The test sample size is 4 g
- Laboratory samples of potatoes and aubergines stored at -20ºC are thawed before processing.
- Transfer the test samples to PP tubes of 50 ml (3.5).

Annex 5. Method of analysis for calystegines in vegetables

5. <u>Extraction and sample preparation procedure for potatoes and aubergines</u>

- A QC potato or aubergine sample is included in each series. This sample is provided by RIKILT.

5.2 Extraction

- Add 37 ml of extraction solution (2.2.1) to the tubes by using a dispenser (3.6).
- Extract for 30 min on a rotary tumbler (overhead shaker) (3.3).
- Centrifuge for 15 min at 3500 rpm (3.4).

5.3 Preparation of extracts for LC-MS/MS analysis

- Take 50 µl of extract and transfer to a 500 µl filtervial (4.8). Add 450 µl mobile phase (2.2.2) and close.
- Take four times 50 µl of extract of the QC potato sample and transfer to four 500 µl filtervials (4.8).
- Add to two QC vials 450 µl mobile phase (2.2.2) and close.
- Add to the other QC vials 100 µl mixed calystegines solution 10 µg/ml (1.2) and 350 µl mobile phase (2.2.2) and close.

NOTE: At this point vials containing the final extracts can be stored in the freezer until analysis.

Part C: LC-MS/MS analysis

6. <u>LC-MS/MS analysis.</u>

6.1 Instrumentation.

A Waters Xevo TQ-S tandem mass spectrometer, operated in positive electrospray mode, in combination with a Waters Acquity UPLC is used (regular HPLC equipment can also be used).

6.2 MS method

The MS methods have been optimised during validation for the Waters TQ-S system. The MS method have been installed on the TQ-S system.

6.2.1 Tune method: SOPA1061.

Tune settings:

- Source temperature: 150°C.
- Desolvation gas temperature: 600°C.
- Desolvation gas flow: 800 L/h.
- Cone gas flow: 150 L/h.
- Cone voltage: 20 V.
- Capillary voltage: 3.0 kV.
- Argon collision gas pressure: 4.0 mbar.

6.2.2 Inlet method: calystegines_EFSA_2016.

HPLC conditions:

Column: Astec Chirobiotic V chiral column, 150 x 2.1 mm, 5 µm, 100Å (11019AST), Sigma-Aldrich

- Column temperature: 35°C.
- Mobile phase A: 5 mM ammonium acetate pH 7.0 (2.2.2).

- Mobile phase B: methanol (2.2.3).
- Flow: 0.30 ml/min.
- Injection volume: 10 µl.
- Total run time: 15 min.
- Gradient (linear):

Time (min)	Mobile phase A (2.2.2)	Mobile phase B (2.2.3)	Flow rate (ml/min)
0.0	100%	0%	0.30
8.0	100%	0%	0.30
8.5	50%	50%	0.225
11.5	50%	50%	0.225
12.0	100%	0%	0.225
15.0	100%	0%	0.30

6.2.3 MS method: calystegines_EFSA_2016.
MS/MS fragmentation conditions used in MRM method:

Compound	$[M+H]^+$ *m/z*	Frag 1 *m/z*	CE eV	Frag 2 *m/z*	CE eV	Frag 3 *m/z*	CE eV	Frag 4 *m/z*	CE eV	RT min
Calystegine A_3	160.1	142.0	10	**125.0**	**15**	98.2	15	79.2	20	6.70
Calystegine A_5	160.1	142.0	10	125.0	15	**81.2**	**15**	79.2	20	6.10
Calystegine B_1	176.1	**140.0**	**10**	123.0	15	112.0	15	95.2	15	3.20
Calystegine B_2	176.1	**140.0**	**10**	97.2	15	95.2	15	67.2	20	2.60
Calystegine B_3	176.1	158.0	10	116.0	15	**98.2**	**15**	67.2	20	4.40
Calystegine B_4	176.1	140.0	10	123.0	15	**112.0**	**15**	95.2	15	4.80

In bold: most intense fragment

6.3 Injection order of series.

- Working standard 1000 ng/ml (2-3x).
- Mobile phase.
- WS series (9 samples).
- Mobile phase.
- QC extracts (4 samples).
- Mobile phase.
- Sample extracts.
- Mobile phase.
- WS series (9 samples).

7. <u>Data analysis and storage</u>

7.1 Calculations

- Calculate the concentration in the extract (ng/ml) by comparing the sum of the peak area in the extract with the calibration curve prepared from the WS series, constructed by means of the least squares method for linear regression.

- Calculate the matrix effect in the QC extracts by:

$$Matrix_{QC} = \left(\frac{QC_{added} - QC_{blank}}{1000} \right) x\ 100\%$$

$Matrix_{QC}$ = matrix effect calculated for QC sample (%)

QC_{added} = Concentration (ng/ml) calculated for the QC extract with added mix of calystegines (1000 ng/ml)

QC_{blank} = Concentration (ng/ml) calculated for the QC extract without added calystegines

- Calculate the concentration in the potato sample in mg/kg fresh weight, by taking into account the 100 fold dilution in the extract and the average matrix effect calculated for the QC samples. Results are not corrected for extraction efficiency.

7.2 Data analysis and storage

- Raw MS data are processed with Waters Quanlynx 4.1 software.
- Quanlynx data are transported automatically to the Report forms in excel format designed for the analysis of TAs in cereal (products).
- Quantification and acceptance of results is conducted according to criteria described in SANTE/11945/2015.
- Raw MS data are stored on the designated data server.

Annex 5. Method of analysis for calystegines in vegetables