EXTERNAL SCIENTIFIC REPORT

Occurrence of Pyrrolizidine Alkaloids in food[1]

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ABSTRACT

A total of 1 105 samples of animal- and plant-derived products, including milk and milk products, eggs, meat and meat products, (herbal) teas and (herbal) food supplements were analysed for the presence of 28 or 35 pyrrolizidine alkaloids (PAs). Samples were collected in supermarkets, retail shops and for a small proportion via internet between January 2014 and April 2015, in six European countries (France, Germany, Greece, Italy, the Netherlands and Spain). The samples comprised 268 milk and milk products (including yoghurt, cheese and infant formula), 205 eggs, 273 meat (including beef, pork and poultry meat, and liver of beef, pork and chicken), 168 teas (including black, green, rooibos, chamomile, peppermint and mixed herbal tea) and 191 food supplements. All samples were analysed by liquid chromatography coupled to tandem mass spectrometry. The limit of quantification depended on the matrix (from ≤ 0.1 µg/L in milk to 5-10 µg/kg in oil-based food supplements) and was considered fit-for-purpose. One or more PAs were detected in 2 % of the animal-derived products, in 91 % of the (herbal) teas and in 60 % of the food supplements. Eleven milk samples (6 %) contained PAs, but the levels were relatively low (between 0.05 and 0.17 µg/L). Only two egg samples contained trace amounts of PAs (0.10-0.12 µg/kg), and no PAs were detected in the other animal-derived products. In contrast, all types of (herbal) teas investigated were found to contain PAs, with a mean concentration of 6.13 µg/L in (herbal) tea infusion (corresponding to 460 µg/kg dry tea). The highest mean concentrations were found in rooibos tea (7.99 µg/L tea infusion) and the lowest in chamomile (3.65 µg/L tea infusion). Occurrence of PAs in food supplements was found to be highly variable, with the highest concentrations present in supplements containing plant material from known PA-producing plants.

KEY WORDS

pyrrolizidine alkaloids, survey, occurrence, milk, eggs, meat, herbal teas, herbal food supplements, LC-MS/MS

DISCLAIMER

Amendment: This external scientific report replaces the original report published on 3 August 2015. With respect to the original external scientific report, corrections were made in pages 22, 78 and 79, in Tables 35, 36, 37 and 38, in Figure 27,

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and in Appendix F, G and J. No changes were made to the abstract, Summary or Conclusions sections. Changes refer to 13 of 191 food supplements that are consumed as herbal infusion for which incorrect levels of pyrrolizidine alkaloids were reported. Additionally, for dry tea samples some minor inconsistencies between Table 35 and Appendices F and G were corrected. The original version is available on request as well as the version showing the changes made.

SUMMARY

Pyrrolizidine alkaloids (PAs) are secondary metabolites produced by a wide variety of plants from the families of *Asteraceae* (tribes of *Senecio, Eupatorium*), *Boraginaceae* (most genera) and *Fabaceae* (*Crotalaria* genus). PAs are regarded as undesirable substances in food and feed, due to their genotoxic and carcinogenic properties, and for that reason have been the subject of two EFSA opinions (EFSA, 2007, 2011). Due to the limited availability of suitable occurrence data in food products, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) recommended that ongoing efforts should be made to collect analytical data on occurrence of PAs and PA *N*-oxides (PANOs) in relevant food commodities. In April 2013 EFSA published a call for proposals to investigate the concentrations of PAs in animal-derived food products including milk and milk products, eggs and meat and meat products, and for plant-derived food products including (herbal) teas and food supplements, across different regions in Europe.

This report describes the outcome of project GP/EFSA/CONTAM/2013/03, 'Occurrence of Pyrrolizidine Alkaloids in food' carried out in accordance with Article 36 of Regulation (EC) No 178/2002, which was designed to obtain representative data on the occurrence of pyrrolizidine alkaloids in Europe, using validated stat-of-the-art analytical methods.

Two in-house validated analytical methods based on liquid-chromatography coupled with tandem mass spectrometry (LC-MS/MS) were used to detect and accurately quantify 35 different PAs in animal-derived samples and 28 different PAs in plant-derived samples at the low performance levels that were required. Limits of detection ranged from 0.03 to 0.05 µg/L in milk, infant formula and yoghurt, from 0.05-0.15 µg/kg in eggs and cheese, from 0.05 to 0.25 µg/kg in meat and liver, from 0.007-0.025 µg/L in (herbal) tea infusion (corresponding to 0.5-2.0 µg/kg in dry tea), from 0.3 to 2.3 µg/kg in dry herbal supplements, from 0.9-3.8 µg/kg in oil-based supplements and from 0.2-0.6 µg/kg in bee product supplements.

A total of 1 105 samples were collected between January 2014 and April 2015 in 6 different European countries and analysed for the presence of PAs. This included 746 samples of animal origin (268 samples of milk and milk products, 205 egg samples and 273 samples of meat and meat products) and 359 samples of plant origin (168 samples of (herbal) teas and 191 herbal food supplements).

Analysis of the animal-derived products revealed occasional low levels of PAs in milk samples (6 %), mostly with single PAs (i.e. jacoline, senkirkine, otosenine, lycopsamine, echimidine, retrorsine) in their free base form. Except for two egg samples, PAs were absent in the milk products, eggs, meat and liver samples analysed.

The analysis of the (herbal) tea samples revealed that a high proportion of (herbal) teas (91 %) contained one or more PAs. The mean concentration for the sum of 28 PAs was 6.13 µg/L tea infusion, with a maximum of 64.0 µg/L. Of the various types of tea, rooibos tea showed the highest concentration (mean PA concentration of 7.99 µg/L), while chamomile tea on average contained the lowest PA concentration (3.67 µg/L). PAs belonging to the senecionine-type (senecionine, retrorsine, seneciphylline) were the most frequently found. The *N*-oxide forms generally were present in higher concentrations than the free base forms.

Food supplements were often contaminated with PAs (60 %), but the concentrations were highly variable. As expected, the highest PA levels were found in herbal food supplements made from plant material of known PA producers. Supplements containing oil-based extracts of PA-producing plants were generally free of PAs. In the food supplements, PAs belonging to the lycopsamine-type

(lypcosamine, intermedine, echimidine) were the most frequently found. PAs were often present as mixtures of free bases and *N*-oxides.

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BACKGROUND AS PROVIDED BY EFSA

The European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) recently assessed the risk for public and animal health related to the presence of pyrrolizidine alkaloids in food and feed.

Pyrrolizidine alkaloids are toxins found naturally in a wide variety of plant species. Over 350 pyrrolizidine alkaloids are known. The main sources are the families *Boraginaceae* (all genera), *Asteraceae* (tribes *Senecionae* and *Eupatoriae*), and *Fabaceae* (genus *Crotalaria*). Numerous plant families express pyrrolizidine alkaloids, some plant species express several pyrrolizidine alkaloids and there are some pyrrolizidine alkaloids that are expressed by several plant species.

In recent years liquid chromatography-mass spectrometry (LC-MS/MS) has become the method of choice for measurement of pyrrolizidine alkaloids. LC-MS/MS is advantageous as it offers low detection limits.

As 1,2-unsaturated PAs are genotoxic and carcinogenic, the CONTAM Panel concluded that it was not appropriate to establish a Tolerable Daily Intake (TDI), and decided to apply the Margin of Exposure (MOE) approach. Considering acute exposure to honey the CONTAM Panel concluded that there is a possible health concern for toddlers and children who are high level consumers.

Therefore, EFSA wishes to launch a call for proposals for a project to investigate the concentrations of 1,2-unsaturated-pyrrolizidine alkaloids in food for human consumption (excluding honey) from different geographic regions in Europe.

TERMS OF REFERENCE AS PROVIDED BY EFSA

This call for proposals aims to obtain representative occurrence data for pyrrolizidine alkaloids in food samples (excluding honey) with particular focus upon the milk and egg categories and meat samples for human consumption but also generating occurrence data for herbal teas and food supplements from different geographic regions in Europe; analysis will be by a validated LC-MS/MS method. The beneficiary shall perform the following tasks, in order to achieve the objectives:

1. To elaborate a protocol for collecting the samples that is in accordance with the Commission Regulation (EC) No 401/2006[2] and that shall take into account the following requirements:

    a. the samples shall be taken from at least 3 different European countries (preferably not from neighbouring countries);

    b. the following food products for human consumption shall be analysed:
        i. at least 200 samples of milk and milk products for human consumption;
        ii. at least 200 samples of eggs and egg products for human consumption;
        iii. the inclusion of at least 300 samples of meat for human consumption;
        iv. at least 150 samples of herbal teas as prepared for ready to drink;
        v. at least 150 samples of herbal food supplements;

2. To collect the samples;

3. To analyse the samples using a validated LC-MS/MS method that complies with the requirements of the Commission Regulation (EC) No 401/2006[2] and that has a sensitivity comparable to methods that have recently been published in the literature;

[2] Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. OJ L70, 9.3.2006, p. 12–34.

4. To prepare a Final External Scientific Report and a database providing the results of the analyses performed for food samples intended for human consumption. The database as well as the Interim and Final External Scientific Reports will be prepared in line with the time schedule reported in 1.4 of the present call for proposals.

The Final as well as the Interim Scientific reports shall be written in English and will follow the template structure provided by EFSA and the EFSA citation standards. The External Scientific Report shall contain the following information: the justification of the choice and the description of the analytical method applied; the validation results of the method for all analysed matrices (similar matrices can be combined for the validation however reasoning must be provided); the description of the sampling procedure applied; the results of the individual samples; common statistical descriptors (e.g. mean, median, standard deviation) of the concentrations; a critical evaluation of the reliability of the submitted data and the related uncertainties, e.g. in the analytical methods, sampling, etc.

The database shall be written in English and shall follow the EFSA Guidance on standard sample description and should be submitted via the Dietary and Chemical Monitoring Unit's (DCM) call for continuous collection of chemical contaminants occurrence data in food and feed. It shall contain the following information: the concentrations of pyrrolizidine alkaloids (particularly 1,2-unsaturated pyrrolizidine alkaloids and *N*-oxide forms) in the analysed samples, associated information describing the sample and the other sample description details specified in the most recent EFSA Guidance on standard sample description.

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- RIKILT – Wageningen UR, Wageningen, the Netherlands

- Federal Institute for Risk Assessment (BfR), Berlin, Germany

- Institute for Research and Technology in Food and Agriculture (IRTA), Monells, Spain

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INTRODUCTION AND OBJECTIVES

1. INTRODUCTION

Pyrrolizidine alkaloids (PAs) are secondary metabolites produced by a wide variety of plants from the families of *Asteraceae* (tribes of *Senecio*, *Eupatorium*), *Boraginaceae* (most genera) and *Fabaceae* (*Crotalaria* genus). PAs are produced by plants as defence compounds against herbivory by insects and mammalians. PAs are regarded undesirable substances in food and feed and have been the subject of two recent EFSA opinions (EFSA, 2007, 2011). In 2011, EFSA concluded that 1,2-unsaturated PAs must be regarded as probable genotoxic carcinogens, for which no tolerable daily intake (TDI) can be established. Alternatively, the margin of exposure (MOE) approach was followed. Based on the available data, a MOE of 1:10 000 was estimated for an exposure of 7 ng/kg body weight (b.w.) per day. For an adult person, weighting 70 kg, this corresponds to a daily exposure of approximately 500 ng PAs. Besides honey, products of animal origin (milk, eggs, meat) and products of herbal origin (tea, supplements) were considered as potential food commodities that could be contaminated with PAs. Analytical data on occurrence of PAs in honey has been provided to EFSA in recent years, but at the moment this survey was started such data was lacking for herbal- and animal-derived products. Since the start of this project some data on the occurrence of PAs in (herbal) teas and supplements have been published elsewhere (Bodi et al., 2014; Griffin et al., 2014; Mathon et al., 2014; Schulz et al., 2015).

1.1. Objective

The main objective of the present study is to provide representative data on the occurrence of PAs in food products of animal and plant origin, which can be used as supporting information to the CONTAM Panel for future exposure and risk assessment on this group of toxins. The objective will be achieved by sampling at least 1 000 food products, with emphasis on animal-derived products, such as milk, egg, and meat products and on plant-derived products, such as herbal teas and supplements. Samples will be collected from different geographic regions in Europe. Analysis will be performed using state-of-the-art LC-MS/MS methodology, which enables detection at the lowest achievable levels. This will increase the detection rate in case PAs are present at trace levels and thus the number of measurements with numerical occurrence data. Analysis will focus on the PAs for which analytical standards are available at the start of the study (29 different commercial standards). This study will fill some important gaps of knowledge that were identified by EFSA in their 2011 assessment of PAs in food and feed.

1.2. Tasks and responsibilities of the partners

An overview of the tasks and responsibilities identified in this study and their distribution among the partner institutions is presented in Table 1.

Table 1: Description of tasks and responsibilities among the partner institutions

	RIKILT	IRTA	BfR
Animal-derived food products			
Collection of samples (according to the sampling plan)	Yes (NL)	Yes (ES, IT, EL)	Yes (DE)
Preparation of aggregate samples	Yes	Yes	Yes
Sample preparation for analysis	Yes	Yes	- (c)
Sample analysis by LC-MS/MS (a)	Yes	- (d)	-
Preparation of QC and stability samples	Yes	-	-
Inter-laboratory performance comparison	Yes	Yes	-
Plant-derived food products			
Collection of samples (according to the sampling plan)	Yes (NL)	Yes (ES, IT, EL)	Yes (DE)
Preparation of aggregate samples	- (e)	- (e)	Yes
Sample preparation for analysis	-	-	Yes
Sample analysis by LC-MS/MS (b)	-	-	Yes
Preparation of QC samples	-	-	Yes
General tasks and responsibilities			
Sampling plan	Yes	-	Yes
SOPs for collection, storage and processing of samples	Yes	-	Yes
Commutability of LC-MS/MS methods and PA standards	Yes	-	Yes
Recording of sample information in SSD format	Yes	Yes	Yes
Reporting of results to EFSA	Yes	-	(Yes) (f)

DE: Germany; EL: Greece; ES: Spain; NL: the Netherlands.
(a): Samples were analysed using a validated in-house RIKILT method.
(b): Samples were analysed using a validated in-house BfR method.
(c): Aggregate samples were shipped to RIKILT for sample preparation and analysis.
(d): Sample extracts were shipped to RIKILT for analysis by LC-MS/MS.
(e): Samples collected were shipped to BfR for preparation of aggregate samples, preparation of sample extracts and sample analysis by LC-MS/MS.
(f): Results were reported by BfR in SSD format and verified by RIKILT before reporting to EFSA.

1.3. Sampling plan

The survey, as stated in the EFSA GP/EFSA/CONTAM/2013/03[1] call, focussed on products of animal origin, such as milk and dairy products, eggs and eggs products and meat from different animal species (bovine, porcine, poultry and special parts of the animals such as liver), as well as products of herbal origin such as (herbal) teas and (herbal) food supplements.

Table 2 shows the proposed sampling plan per food category based on the specifications given in the EFSA call. Within the scope specified by EFSA, the starting point with respect to the sampling strategy was objective sampling. Therefore the sampling plan was largely consumption oriented. The proportion of organic samples for each item was aimed to be between 10 and 15 %.

The survey was divided in two sampling periods. During the first sampling period (January 2014-June 2014) the following products were collected: all (herbal) teas and infant formula (milk powder) samples, around 50 % of the eggs and milk samples, and around 65 % of beef, pork and poultry meat samples. During the second sampling period (September 2014-April 2015) the following products were collected: the remaining samples of meat, milk and eggs, all samples of cheese, yoghurt, liver and the (herbal) supplements. Some modifications were made to the sampling plan in September 2014, when it was agreed, in view of the preliminary results from the first sampling period, to increase the total number of milk samples from 200 to 250 and to reduce the total number of meat samples from 300 to 250.

Table 2: Summary of the proposed sampling plan

	Total number of samples	Number of organic samples	Sampling period	
			Jan 2014 - Jun 2014	Sep 2014 - Apr 2015
All animal-derived food products	**700**	**75**	**350**	**350**
Milk and milk products [(a)]	**250**	**25**	**100**	**150**
Pasteurised and UHT milk (skimmed, semi-skimmed, whole milk)	175	20	75	100
Fermented milk products (yogurt, pudding, quark)	25	-	-	25
Cheese (soft, hard)	25	-	-	25
Milk powder (infant formula)	25	5	25	-
Eggs and egg products [(b)]	**200**	**20**	**100**	**100**
Fresh eggs	200	20	100	100
Meat and meat products [(c)]	**250**	**30**	**150**	**100**
Beef meat	75	10	50	25
Pork meat (filet)	75	10	50	25
Poultry meat (chicken breast filet)	75	10	50	25
Liver (beef, pork, poultry)	30	-	-	30
All plant-derived food products	**300**	**40**	**150**	**150**
(Herbal) teas	**150**	**20**	**150**	-
Black tea	30	4	30	-
Green tea	20	3	20	-
Rooibos tea	20	3	20	-
Chamomile tea	30	4	30	-
Peppermint/poleo mint tea	30	4	30	-
Mixed herbal tea	20	3	20	-
(Herbal) food supplements	**150**	**20**	-	**150**
Supplements based on plants not known to produce PAs	75	10	-	75
Supplements based on plants known to produce PA**s**	50	7	-	50
Supplements containing bee products	25	3	-	25

(a): In the original call text it was envisioned to collect a total of 200 samples of milk and milk products. In September 2014, in view of the preliminary results from the first sampling period, it was agreed to raise the number to 250, by increasing the number of pasteurised and UHT milk samples.

(b): In the original call text it was envisioned to collect 10 samples of egg powder. Due to the fact that this product was not available in regular retail shops and supermarkets, it was agreed to exclude this item from the sampling.

(c): In the original call text it was envisioned to collect a total of 300 samples of meat and meat products. In September 2014, in view of the preliminary results from the first sampling period, it was agreed to lower the number to 250, by decreasing the number of beef, pork and poultry meat samples.

Table 3 shows the proposed sampling plan per country. The number of samples per country was based on the human consumption of these products in the different regions within Europe. It was considered of great importance that the samples collected were representative of the situation in the EU with regard to the domestic production of the different target products and to the consumption habits in the different regions. Six countries covering the south, the northwest and central Europe were sampled, which represented around 55 % of the European Union population. Furthermore, the sampling countries are included in different dietary groups, according to the classification of the World Health Organization (WHO, 2012).

Table 3: Proposed sampling plan per country

Responsible	Sampling country	Total	Milk (products)	Eggs (products)	Meat (products)	Herbal teas	Herbal food supplements
RIKILT	Netherlands (NL)	220	70	50	40	30	30
BfR	Germany (DE)	230	50	40	40	50	50
IRTA	Spain (ES)	225	50	40	75	30	30
	France (FR)	120	30	25	35	15	15
	Italy (IT)	120	30	25	35	15	15
	Greece (EL)	85	20	20	25	10	10
Total		1 000	250	200	250	150	150

MATERIALS AND METHODS

2. COLLECTION, TRANSPORT AND STORAGE OF THE SAMPLES

The samples were collected in supermarkets, shops and other retail outlets. A limited number of (herbal) food supplements (15%) were purchased from webshops. The sampling was conducted taking as guidance the methods of sampling for official control laboratories described in Commission Regulation (EC) No 401/2006.

As described in Commission Regulation (EC) No 401/2006[2], for each product three items with the same expiration date and the same lot number were collected. The combined amount of product collected should be sufficient to prepare an aggregate sample of at least 1 kg in case of meat and milk or 100 g in case of teas. For eggs, the three items contained each at least 6 eggs.

The sampling of the (herbal) tea products was performed taking as guidance epigraph E.4 of Commission Regulation (EC) No 401/2006, describing the sampling methods for spices. Three incremental samples from the (sub)lot were taken to form an aggregate. One package of tea (bags) had a sample size of approximately 30-60 g. The incremental samples were combined and homogenised to form a final aggregate of at least 100 g.

(Herbal) food supplements were collected according to SANCO/10556/2013, Rev. 1 (amending Regulation (EC) No 401/2006). In the epigraph M the Regulation describes the sampling methods for citrinin in food supplements based on rice fermented with red yeast *Monascus purpureus*. The sampling procedure was based on the supposition that these food supplements are marketed in retail packages containing usually 30 to 120 capsules per retail package. Depending on the lot size (number of retail packages per lot) the number of retail packages to be taken for sample is defined. For lot sizes between 1-50 packages, a single package was taken, and for lot sizes between 51-250 packages two packages were taken. In both cases all capsules were combined and homogenised to form a final aggregate sample.

The purchased products were transported and stored at the usual temperature of storage of the product in the retail shop, e.g. at cooled condition (4-6 °C) for perishable products such as fresh milk and meat products. Products with an extended shelf-life such as UHT treated milk or eggs were either stored at room temperature or under cooled conditions. The products were not frozen before preparation of the aggregate sample.

All relevant information of the sample (as described on the product label) as well as the place and date of collection was recorded in the EFSA Standard Sample Description (SSD) form. The original

packing and/or labels were kept as a back-up of the available product information. Alternatively, or additionally, scans and/or photos were taken of the sample for the same purpose.

3. PREPARATION OF AGGREGATE AND SUB-SAMPLES

The aggregates and sub-samples were prepared as soon as possible after collection, and always before the expiration date.

3.1. Animal-derived food products

An aggregate sample of ca. 1 kg was prepared by combining equal amounts of the three identical collected items.

3.1.1. Milk and milk products

Milk. Prior to measuring by volume the required amount, the purchased items were thoroughly shaken. Then, the three fractions were combined in an appropriate container, e.g. a 1 L polypropylene bottle with screw cap, and thoroughly homogenised.

After homogenization, aliquots (40 mL) of the aggregate milk samples were transferred into three polypropylene tubes of 50 mL. The respective three sub-samples and the aggregate sample were appropriately coded and stored at -20 °C until analysis or shipment.

Infant formula. Infant formula (milk powder) was reconstituted according to the instructions on the product label. For preparation of 330 mL reconstituted infant formula milk, 45-50 g of infant formula powder was thoroughly mixed with 300 mL boiling water. Infant formula milk was further processed as described for milk.

Yoghurt. Prior to measuring by weight the required amount, the purchased items were thoroughly mixed. Then, the three fractions were combined in an appropriate container, e.g. a 1 L polypropylene bottle with screw cap, and thoroughly homogenised.

After homogenization, aliquots (40 g) of the aggregate yoghurt samples were transferred into three polypropylene tubes of 50 mL. The respective three sub-samples and the aggregate sample were appropriately coded and stored at -20 °C until analysis or shipment.

Cheese. Hard cheese was ground with a cheese mill and soft cheese was ground cryogenically with liquid nitrogen. After homogenization, three sub-samples (approx. 40 g) of the aggregate ground cheese were transferred into polypropylene tubes of 50 mL. The respective three sub-samples and the aggregate sample were appropriately coded and stored at -20 °C until analysis or shipment.

3.1.2. Eggs

From each of the three egg packages collected, an equal number of eggs (i.e. six) was selected. The shell of the eggs was crushed and the content (yolk and white) were poured into an appropriate container. The shells were discarded. The aggregate sample was then homogenised with a hand-held immersion blender, which was operated at medium speed for ca. 2 minutes until the sample started foaming and no differences in colour were observed. Three aliquots (around 40 mL) of the homogenised aggregate egg sample were poured into polypropylene tubes of 50 mL. The respective three sub-samples and the aggregate sample were appropriately coded and stored at -20 °C until analysis or shipment.

3.1.3. Meat and liver (bovine, porcine, poultry)

Meat. Three portions of each meat type (bovine, porcine and poultry) of approximately the same weight were taken and, after removing tendons and/or fat, they were cut into small cubes. The cubes were ground and homogenised with a meat-mincing device with sufficient capacity to grind 1 kg of meat. After homogenization, three portions (approx. 40 g of ground meat) of the aggregate sample were transferred to polypropylene tubes of 50 mL. The respective three sub-samples and aggregate sample were appropriately coded and stored at -20 °C until analysis or shipment.

Liver. Three portions of each liver type (bovine, porcine and poultry) of approximately the same weight were taken and homogenised with a hand-held immersion blender for approx. 3 minutes. Three aliquots (40 mL) of each of the aggregate samples were transferred to polypropylene tubes of 50 mL. The respective three sub-samples and aggregate sample were appropriately coded and stored at -80 °C until analysis or shipment.

3.2. Plant-derived food products

3.2.1. (Herbal) teas

An aggregate sample of at least 100 g of each (herbal) tea was prepared by combining equal amounts of three identical collected items. One package of tea (bags) had a typical sample size of approximately 30-60 g. After removing the bags, the aggregate tea sample was mixed with dry ice (at a mass ratio of 2:1). The mixture was allowed to stand for about 3 minutes while stirring repeatedly. The frozen sample was ground to a particle size of 500 µm using an ultra-centrifugal mill (ZM 200, Retsch, Haan, Germany). The aggregate sample was homogenised by overhead-shaking for 2 hours. Finally, three sub-samples of the aggregate sample were transferred to polypropylene tubes of 50 mL. The respective three sub-samples and aggregate sample were appropriately coded and stored at room temperature until analysis.

3.2.2. (Herbal) food supplements and bee products

Dry food supplements and bee pollen products. For each supplement the respective sample weight per single dosage form (e.g. tablet or capsule) was determined and recorded. An aggregate sample was prepared by combining all tablets or capsules of the package. In the case of encapsulated food supplements, the coatings were removed before homogenisation. The aggregate samples were mixed with dry ice (at a mass ratio of 2:1). The mixture was allowed to stand for about 3 minutes while stirring repeatedly. The frozen sample was ground to a particle size of 500 µm using an ultra-centrifugal mill (ZM 200, Retsch, Haan, Germany). The aggregate samples were homogenised by overhead-shaking for 30 minutes. Finally, three sub-samples of the aggregate sample were transferred to polypropylene tubes of 50 mL. The respective three sub-samples and aggregate sample were appropriately coded and stored at room temperature until analysis.

Oil-based food supplements. For each supplement the respective sample weight per single dosage form (capsule) was determined and recorded. For the preparation of the aggregate samples, the oil was removed from the capsules using an appropriate syringe and subsequently combined. The aggregate simple was homogenised by overhead-shaking for 30 minutes. Finally, three sub-samples of the aggregate sample were transferred to polypropylene tubes of 50 mL. The respective three sub-samples and aggregate sample were appropriately coded and stored at room temperature until analysis.

4. STANDARDS AND QUALITY CONTROL (QC) MATERIALS

4.1. Standards

A total of 39 standards of PAs were available for this study. The standards were obtained either from various suppliers (29 were commercially available), had previously been isolated from plant material (7 standards), or had previously been (in-house) synthesised (3 standards). Four standards isolated from plant material had been received as gifts from other research institutes as specified in Table 4. Some standards were available only in very small amounts and could therefore not be exchanged between the partners. Some standards were available but were not included in the methods, because of insufficient separation from structural analogues, as will be discussed in Section 6 in more detail. The chemical structures of the PA standards included in this study can be found in Appendix A.

Table 4: Pyrrolizidine alkaloid (PA) standards used in the study

PAs [(a)]	Laboratory	Origin standard	Supplier/source	Batch	Purity [(b)]
Em	BfR	Commercial	Phytolab 89553	6603	96.72 %
	RIKILT	Commercial	Phytolab 89553	9008	95.20 %
EmNO	BfR	Synthesised	RIKILT		95 %
	RIKILT	Synthesised	In-house		95 %
Er	BfR	Commercial	Phytolab 83432	8518	98.92 %
	RIKILT	Commercial	Phytoplan 6218	12110201	99.93 %
ErNO	BfR	Commercial	Phytolab 83433	8722	98.42 %
	RIKILT	Commercial	Phytoplan 6221	12110401	99 %
Eu	BfR	Commercial	Phytolab 83237		99.90 %
	RIKILT	Commercial	Phytoplan 6214	12050201	97 %
EuNO	BfR	Commercial	Phytolab 83238	7159	99.32 %
	RIKILT	Commercial	Phytoplan 6215	12040601	97 %
Fs	BfR	Not in method [(*)]	-		
	RIKILT	Isolated	PRISNA		95 %
He	BfR	Commercial	Latoxan L6007	210.120	98 %
	RIKILT	Commercial	Latoxan L6007	210.120	98 %
HeNO	BfR	Commercial	Oskar Tropisch 0054	12030701	97.96 %
	RIKILT	Commercial	Phytoplan 6213	12030701	97 %
Id	BfR	Not in method [(**)]	Phytolab 83234		
	RIKILT	Not in method [(**)]	Phytolab 83234	7598	98.25 %
IdNO	BfR	Not in method [(**)]	Phytolab 83235		
	RIKILT	Commercial	Phytolab 83235	7162	94.77 %
Ir	BfR	Not in method [(*)]	-		
	RIKILT	Isolated	UNICAMP [(c)]		90 %
IrNO	BfR	Not in method [(*)]	-		
	RIKILT	Isolated	UNICAMP [(c)]		90 %
Im	BfR	Commercial	Phytolab 82424	4871	97.35 %
	RIKILT	Not in method [(**)]	Phytolab 82424	4871	97.35 %
ImNO	BfR	Commercial	Phytolab 83446	8852	100 %
	RIKILT	Not in method [(**)]	Phytolab 83446	8852	
Jb	BfR	Commercial	Phytolab 83434	8516	100 %
	RIKILT	Commercial	Phytoplan 6219	13090402	99.16 %
eJb	BfR	Not in method [(*)]	-		
	RIKILT	Synthesised	Mercachem		95 %
JbNO	BfR	Commercial	Phytolab 83435	8721	96.5 %
	RIKILT	Commercial	Phytolab 83435	8721	96.5%
Jl	BfR	Not in method [(*)]	-		

PAs [a]	Laboratory	Origin standard	Supplier/source	Batch	Purity [b]
	RIKILT	Isolated	PRISNA		90 %
Lc	BfR	Commercial	Oskar Tropisch 0019	11110401	97.96 %
	RIKILT	Commercial	Phytolab 89726	3652	98 %
LcNO	BfR	Commercial	Oskar Tropisch 1284	11121301	99.60 %
	RIKILT	Commercial	Phytoplan 6211	11121301	96 %
Ly	BfR	Commercial	Phytolab 89726	4870	95.52 %
	RIKILT	Commercial	Phytolab 89726	3652	87 %
LyNO	BfR	Commercial	Phytolab 83447	8723	97.60 %
	RIKILT	Commercial	Phytolab 83447	8723	97.50 %
Mc	BfR	Commercial	Roth 3418	34573847	99 %
	RIKILT	Commercial	Phytolab 89251	8573	98.90 %
McNO	BfR	Commercial	Phytolab 82629	4687	99.42 %
	RIKILT	Commercial	Phytolab 82629	4687	99.42 %
Ot	BfR	Not in method (*)	-		
	RIKILT	Isolated	Phytolab	4016	95 %
Re	BfR	Commercial	Sigma R0382	034K1121	97 %
	RIKILT	Commercial	Phytoplan 6203	11030712	98.90 %
ReNO	BfR	Commercial	Phytolab 82630	4722	99.75 %
	RIKILT	Commercial	Phytolab 82630	6115	99.90 %
Rd	BfR	Not in method (*)	-		
	RIKILT	Isolated	NTP (c)		95 %
RdNO	BfR	Not in method (*)	-		
	RIKILT	Isolated	NTP (c)		95 %
Sn	BfR	Commercial	Roth 2261	40790110	95 %
	RIKILT	Commercial	Phytoplan 6202	12100121	99.40 %
SnNO	BfR	Commercial	Phytolab 82631	4723	95.48 %
	RIKILT	Commercial	Phytolab 82631	6238	100 %
Sp	BfR	Commercial	AppliChem A2072	09110104	100 %
	RIKILT	Commercial	Phytolab 89275	4333	99.80 %
SpNO	BfR	Commercial	Phytolab 82632	4724	98.25 %
	RIKILT	Commercial	Phytolab 82632	4724	98.30 %
Sv	BfR	Commercial	Phytolab 83436	8520	97.93 %
	RIKILT	Commercial	Phytoplan 6206	12100301	96.03 %
SvNO	BfR	Commercial	Phytolab 83437	8521	98.45 %
	RIKILT	Commercial	Phytoplan 6220	13080702	96.40 %
Sk	BfR	Commercial	Phytolab 89274	1761	97.16 %
	RIKILT	Commercial	Phytoplan 6205	11080510	98.20 %
Td	BfR	Commercial	Latoxan L6049	508	100 %
	RIKILT	Commercial	Latoxan L6049	508	100 %
TdNO	BfR	Not in method (*)	-		
	RIKILT	Synthesised	In-house		100 %

(*): PA was not available as standard in this lab and was therefore not included in the scope of the method.

(**): PA was available as standard, but was not included in the scope of the method due to co-elution with a structurally related PA.

(a): Em: echimidine; EmNO: echimidine-*N*-oxide; Er: erucifoline; ErNO: erucifoline-*N*-oxide; Eu: europine; EuNO: europine-N-oxide; Fs: florosenine; He: heliotrine; HeNO: heliotrine-*N*-oxide; Id: indicine; IdNO: indicine-*N*-oxide; Ir: integerrimine; IrNO: integerrimine-*N*-oxide; Im: intermedine; ImNO: intermedine-*N*-oxide; Jb: jacobine; eJb: *epi*-jacobine; Jl: jacoline; Lc: lasiocarpine; LcNO: lasiocarpine-*N*-oxide; Ly: lycopsamine; LyNO: lycopsamine-*N*-oxide; Mc: monocrotaline; McNO: monocrotaline-*N*-oxide; Re: retrorsine; ReNO: retrorsine-*N*-oxide; Rd: riddelliine; RdNO: riddeliine-*N*-oxide; Sn: senecionine; SnNO: senecionine-*N*-oxide; Sp: seneciphylline; SpNO: seneciphylline-*N*-oxide; Sv: senecivernine; SvNO: senecivernine-*N*-oxide; Sk: senkirkine; Td: trichodesmine; TdNO: trichodesmine-*N*-oxide.

(b): When no purity was stated by the supplier, a purity of 100 % was assumed.

(c): Obtained as gift.

4.2. Quality control (QC) materials

4.2.1. Preparation of matrix-matched (recovery) standards (MM(R)S) and quality control samples (QC) for the analysis of animal-derived products

Blank materials were prepared from milk, eggs, beef meat, pork meat and poultry meat purchased from local supermarkets and were homogenised with the appropriate apparatus. An overview of the selected materials is given in Table 5. The materials selected did not contain PAs (<LOD). For each matrix, the same batch of material was used for the preparation of the matrix-matched standards (MMS) and the matrix-matched recovery standards (MMRS). Two other (smaller) batches of blank material were used for the preparation of two different quality control (QC1 and QC2) samples. Combined sets of the prepared MMS, MMRS and the two QC samples were to be included in each series of samples (see below).

In addition, for each of the five matrices, samples were prepared for check of the stability of PAs under storage conditions. To this end, the blank materials that were used for the preparation of the MMS and MMRS samples were also used to prepare stability samples (Table 5).

Table 5: Blank materials used as matrix-matched standards (MMS), matrix-matched recovery standards (MMRS), stability samples and as quality control samples (QC) for the analysis of animal-derived products

Matrix	MMS, MMRS, stability	QC1	QC2
Milk	Semi-skimmed milk, pasteurised	Whole milk, pasteurised	Skimmed milk, UHT
Eggs	Free-range eggs	Omega 3 eggs	Free-range eggs
Meat, beef	Steak (beef)	Ground beef (low fat)	Lean steak (beef)
Meat, pork	Pork filet	Pork filet [(a)] (pieces)	Pork filet
Meat, poultry	Chicken breast filet	Turkey breast filet	Chicken thigh filet

(a): This pork filet material was bought already cut in filets while the other materials used to prepare MMRS and QCs were bought as one piece.

Based on the number of samples to be analysed by IRTA and RIKILT, the number of MM(R)S/QC sample sets required for the project was estimated for the duration of the project. An average series of samples was estimated to contain approximately 20 individual samples. Training and back-up series were also included. A summary of the total number of MM(R)S/QC samples is shown in Table 6.

Table 6: Total number of MM(R)S/QC sample sets prepared for the analysis of animal-derived products

Matrix	IRTA 1st period	IRTA 2nd period	RIKILT 1st period	RIKILT 2nd period	Total
Milk	5	3	5	2	15
Eggs	6	4	5	3	18
Meat, beef	5	3	2	2	12
Meat, pork	4	3	3	2	12
Meat, poultry	5	3	2	2	12
Total	25	16	17	11	69

Each set of MM(R)S/QC samples contained 7 MMS, 3 MMRS, 2 QC1 and 2 QC2 samples. The MMS/QC samples were fortified at the levels specified in Table 7. The MMRS remained unspiked.

Table 7: Spiking levels (µg/L for milk, µg/kg for eggs and meat) for the MMS/QC/stability samples used for animal-derived products

Matrix	No. of sets	MMS	QC1	QC2	Stability[(a)]
Milk	15	0, 0.1, 0.25, 0.5, 1, 2.5, 5	0, 2.5	0, 2.5	2.5
Eggs	18	0, 0.25, 0.5, 1, 2.5, 5, 10	0, 10	0, 10	10
Meat, beef	12	0, 0.25, 0.5, 1, 2.5, 5, 10	0, 5	0, 5	5
Meat, pork	12	0, 0.25, 0.5, 1, 2.5, 5, 10	0, 5	0, 5	5
Meat, poultry	12	0, 0.25, 0.5, 1, 2.5, 5, 10	0, 5	0, 5	5

(a): Five sets of stability samples were produced for each matrix.

Samples for stability tests of PAs under storage conditions were prepared in 5 sets of 5 samples each, of which 2 sets were stored at -80 °C and 3 sets were stored at -20 °C. The stability samples were fortified at the levels specified in Table 7. Halfway the project, after 7 months (September 2014), for each matrix one set of 5 samples stored at -20 °C and one set of 5 samples stored at -80 °C were analysed to assess the PA analyte stability in matrix under medium term storage conditions at -20 °C (compared to storage at -80 °C). The stability of PAs in matrix under long-term storage conditions at -20 °C (compared to storage at -80 °C) was checked at the end of the project, after 15 months (May 2015). At that time for each matrix a second set of 5 samples stored at -20 °C and a second set of 5 samples stored at -80 °C were analysed.

4.2.2. Preparation of matrix-matched calibration samples and recovery samples for the analysis of plant-derived products

(Herbal) teas. Herbal teas, rooibos, black and green teas which were shown to be free of PAs by previous analyses were used as blank materials for the preparation of MMS solutions. Herbal tea materials were purchased from supermarkets and local pharmacies. For the evaluation of matrix effects, that may differ between types of herbal teas, a pragmatic approach was applied for the preparation of the blank material, by mixing peppermint, chamomile, caraway and fennel tea. This blank material was used for the analysis of herbal tea and rooibos tea samples.

For the quantification of PA in black and green tea infusion samples, blank black tea material and blank green tea material, respectively, were used for the preparation of matrix-matched calibration samples.

Blank tea extracts were prepared according to the sample preparation procedure described in Section 5.2.1. These blank extracts were spiked (after SPE concentration) using a multi-PA standard solution, resulting in a 9-point set of matrix-matched calibration samples as described in Table 8.

Table 8: Levels for matrix-matched calibration and recovery samples for (herbal) teas

Matrix	Series no.	MMS in sample extract for LC-MS/MS (µg/L)	MMS corresponding to tea infusion (µg/L)	Recovery sample for tea infusion (µg/L)
(Herbal) tea	1-9	1, 5, 10, 25, 50, 75, 100, 125, 150	0.03, 0.13, 0.27, 0.67, 1.33, 2.67, 3.33, 4.00	0.27

For each series of samples, a recovery sample was prepared by spiking blank tea infusion with a mixture of PA standards at 0.27 µg/L (corresponding to 20 µg/kg dry product). The recovery sample was analysed in the same way as the (herbal) tea samples (Table 8).

(Herbal) food supplements. Due to expected differences in matrix effects during LC-MS analysis and slight differences in the sample preparation procedure, (herbal) food supplements were divided into four sub-groups: (i) dry supplements, (ii) oil-based supplements and (iii) supplements containing bee products. The fourth group (iv) comprised dry supplements which were labelled to be prepared as tea infusions. These samples were treated as described above for (herbal) teas.

Individual samples for each sub-group which were shown to be free of PA by previous analyses were used for the preparation of matrix-matched standard solutions. The blank matrix for dry supplements was a mixture of equal proportions of fenugreek (*Trigonella foenum-graecum*), milk thistle (*Silybum marianum*) and chebulic myrobalan fruit (*Terminalia chebula*). As blank matrix for oil-based products sun flower oil was used and for bee products a mixture of equal proportions of propolis, royal jelly and pollen was taken as blank material.

Blank sample extracts of the four matrix sub-groups were prepared according to the sample preparation procedure described in Section 5.2.2. For dry supplements and supplements containing bee pollen products, the blank extracts were fortified with a multi-PA standard solution, resulting in a 9-point set of matrix-matched calibration samples with levels summarised in Table 9. For oil-based supplements a five point calibration was applied (Table 9).

Samples that contained analyte concentrations exceeding the calibration range were proportionally diluted with blank sample extract and reanalysed in a separate batch.

Table 9: Levels for matrix-matched calibration and recovery samples for the analysis of food supplements

Matrix	Series no.	MMS in sample extract for LC-MS/MS (µg/L)	MMS corresponding to starting material (µg/kg)	Recovery sample in starting material (µg/kg)
Dry food supplements	1-7	1, 5, 10, 25, 50, 75, 100, 125, 150	8, 40, 80, 200, 400, 600, 800, 1 000, 1 200	80
Supplements containing bee products	8,9	1, 5, 10, 25, 50, 75, 100, 125, 150	8, 40, 80, 200, 400, 600, 800, 1 000, 1 200	80
Oil-based food supplements	10	1, 5, 10, 25, 50	1.2, 6, 12, 30, 60	6

For each series of samples a recovery sample was prepared by spiking blank material with a mixture of PA standards (Table 9). As comparatively high concentrations were expected for supplements derived from dried plant products and bee pollen, a spiking level at 80 µg/kg was chosen. Since low concentrations were expected in oil-based supplements, those recovery samples were spiked at 6 µg/kg. The recovery sample was analysed in the same way as the food supplement samples.

5. SAMPLE PREPARATION

5.1. Animal-derived food products

5.1.1. Milk and milk products

Milk, yoghurt and reconstituted infant formula milk samples were thawed in a water bath at 37 °C and homogenised by shaking by hand. Aliquots of 3 mL were transferred to polypropylene tubes of 50 mL and 30 µL of internal standard solution (*epi*-jacobine at 1 000 ng/mL in methanol) was added. Twenty-seven mL of formic acid solution (0.2 %) and 15 mL hexane were added to the tubes. The samples were extracted for 30 minutes on a rotary tumbler and then centrifuged for 15 minutes at 3500 rpm. The hexane top layer and (most of) the solid middle layer (containing mostly fat and non-soluble proteins) were removed by suction. Concentrated ammonia (25 %) was added to adjust the pH of the solution to 9-10. The samples were centrifuged for another 15 minutes at 3 500 rpm.

Fifteen mL of the remaining aqueous extract was used for further clean-up by solid phase extraction (SPE) over a StrataX 200 mg, 6cc cartridge (Phenomenex, Torrance, CA, USA). The cartridges were conditioned with 6 mL methanol, followed by 6 mL ammonia solution (0.1 %). The cartridges were loaded with 15 mL of extract, washed with 6 mL ammonia solution (0.1 %) and dried under vacuum (using a vacuum manifold) for 5-10 minutes. PAs were eluted from the cartridges with 5 mL of methanol. The eluates were dried under a nitrogen stream in a warmed water bath (50 °C, TurboVap, Zymark, Uppsala, Sweden) and reconstituted in 500 µL of methanol/water (10/90, v/v). The reconstituted sample extracts were filtered using 0.45 µm PTFE 500 µL filtervials (UniPrep, Whatman, Maidstone, UK).

Cheese was thawed by standing at room temperature. Of each sample two portions of 3 g were transferred to polypropylene tubes of 50 mL and 30 µL of internal standard solution (*epi*-jacobine at 1 000 ng/mL in methanol) was added. To one of the portions 300 µL PAs mix (100 ng/mL) was added (equivalent to 10 ng/g cheese) and the tube was vortexed for 20 seconds. Extraction and SPE clean-up was performed as described for milk.

5.1.2. Eggs

Egg samples were thawed in a water bath at 37 °C and homogenised by shaking by hand. Aliquots of 3 mL were transferred to polypropylene tubes of 50 mL and 30 µL of internal standard solution (*epi*-jacobine of 1 000 ng/mL in methanol) was added. Thirty mL of formic acid solution (0.2 %) and 15 mL hexane were added to the tubes. The samples were extracted for 30 minutes on a rotary tumbler and then centrifuged for 15 minutes at 3 500 rpm. The hexane top layer and (most of) the solid middle layer (containing mostly fat and non-soluble proteins) were removed by suction. Concentrated ammonia (25 %) was added to adjust the pH of the solution to 9-10. The samples were centrifuged for another 15 minutes at 3 500 rpm.

Five mL of the remaining aqueous extract was used for further clean-up by SPE over a StrataX 200 mg, 6cc cartridge (Phenomenex, Torrance, CA, USA). The cartridges were conditioned with 6 mL methanol and 6 mL ammonia solution (0.1 %). The cartridges were loaded with 5 mL of extract, washed with 6 mL ammonia solution (0.1 %) and dried under vacuum (using a vacuum manifold) for 5-10 minutes. PAs were eluted from the cartridges with 5 mL of methanol. The eluates were dried under a nitrogen stream in a warmed water bath (50 °C, TurboVap, Zymark, Uppsala, Sweden) and reconstituted in 500 µL of methanol/water (10/90, v/v). The reconstituted sample extracts were filtered using 0.45 µm PTFE 500 µL filtervials (UniPrep, Whatman, Maidstone, UK).

5.1.3. Meat and liver

Ground meat samples were thawed overnight. Portions of 3 g were transferred to polypropylene tubes of 50 mL and 30 µL of internal standard solution (*epi*-jacobine of 1 000 ng/mL in methanol) was added. Thirty mL of formic acid solution (0.2 %) and 15 mL hexane were added to the tubes. The samples were extracted for 30 minutes on a rotary tumbler and then centrifuged for 15 minutes at 3 500 rpm. The hexane top layer and (most of) the solid middle layer (containing mostly fat and non-soluble proteins) were removed by suction. Concentrated ammonia (25 %) was added to adjust the pH of the solution to 9-10. The samples were centrifuged for another 15 minutes at 3 500 rpm.

Five mL of the remaining aqueous extract was used for further clean-up by SPE over a StrataX 200 mg, 6cc cartridge (Phenomenex, Torrance, CA, USA). The cartridges were conditioned with 6 mL methanol and 6 mL ammonia solution (0.1 %). The cartridges were loaded with 5 mL of extract, washed with 6 mL ammonia solution (0.1 %) and dried under vacuum (using a vacuum manifold) for 5-10 minutes. PAs were eluted from the cartridges with 5 mL of methanol. The eluates were dried under a nitrogen stream in a warmed water bath (50 °C, TurboVap, Zymark, Uppsala, Sweden) and reconstituted in 500 µL of methanol/water (10/90, v/v). The reconstituted sample extracts were filtered using 0.45 µm PTFE 500 µL filtervials (UniPrep, Whatman, Maidstone, UK).

Liver was thawed by standing at room temperature. Of each sample two portions of 3 g were transferred to polypropylene tubes of 50 mL and 30 µL of internal standard solution (*epi*-jacobine at 1 000 ng/mL in methanol) was added. To one of the portions, 300 µL PAs mix (100 ng/mL) was added (equivalent to 10 ng/g liver) and the tube was vortexed for 20 s. Extraction and SPE clean-up was performed as described for meat.

5.2. Plant-derived food products

5.2.1. (Herbal) teas

(Herbal) tea samples were mixed with dry ice (at a mass ratio of 2:1). The mixture was allowed to stand for about 3 minutes while stirring repeatedly. The frozen sample was ground to a particle size of 500 µm using an ultra-centrifugal mill (ZM 200, Retsch, Haan, Germany). The extraction procedure was based on the protocol for the preparation of ready-to-drink products described in ISO 3103 [Tea – Preparation of liquor for use in sensory tests] (ISO, 1980). A 2 g amount of tea in a tea infusion bag was placed in a 250 ml beaker and extracted with 150 mL of boiling water. Infusion was steeped for 5 minutes after which the tea bag was removed. After cooling down, the infusion was filtered through a fluted filter paper. An aliquot of 50 mL was used for further clean-up by SPE.

The SPE clean-up was carried out with reversed phase C18 SPE cartridges (Discovery DSC-C18 500 mg/6 mL, Supelco, Bellefonte, PA, USA), which were conditioned with 5 mL of methanol and 5 mL of water. Then, the cartridges were loaded with 50 mL of the (herbal) tea infusion, washed with 6 mL of water and dried under vacuum (using a vacuum manifold) for 5-10 minutes. PAs were eluted from the cartridges in two steps with 5 mL each of methanol or 2.5 % (1.4 M) ammonia in methanol in the case of black and green tea samples. The combined eluates were dried under a nitrogen stream in a warmed water bath (50 °C, TurboVap, Biotage, Uppsala, Sweden) and reconstituted in 1 mL of methanol/water (5/95, v/v). The reconstituted sample extracts were filtered through centrifuge filters (Nylon, 0.2 µm, VWR, Darmstadt, Germany) at 13 000 x g before LC-MS/MS analysis.

5.2.2. (Herbal) food supplements and bee products

For dry food supplements and food supplements containing bee pollen products, an amount of 0.5 g was extracted with 20 mL of aqueous sulphuric acid solution (0.05 M) by ultra-sonication (15 minutes). The supernatant was decanted after centrifugation. Extraction was repeated and combined supernatants were brought to pH 6-7 with diluted ammonia solution and passed through a folded filter paper. An aliquot of 10 mL was used for further clean-up by SPE.

The SPE clean-up was carried out with reversed phase C18 SPE cartridges (Discovery DSC-C18 500 mg/6 mL), which were conditioned with 5 mL of methanol and 5 mL of water. Then, the cartridge was loaded with 10 mL of the sample extract, washed with 8 mL of water and dried under vacuum (using a vacuum manifold) for 5-10 minutes. PAs were eluted from the cartridge in two steps with 5 mL each of methanol. The combined eluates were dried under a nitrogen stream in a heated water bath (50 °C, TurboVap) and the drying residue was reconstituted in 1 mL of methanol/water (5/95, v/v). The reconstituted sample extract was filtered through centrifuge filters (Nylon, 0.2 µm) before LC-MS/MS analysis.

For oil-based food supplements, an amount of 5.0 g was extracted with 15 ml of 0.05 M sulphuric acid in methanol by overhead shaking (15 minutes). The supernatant was decanted after centrifugation. Extraction was repeated and an aliquot of 25 mL of combined supernatants was used for further clean-up by SPE.

The SPE clean-up was carried out using cation exchange SPE cartridges (Bond Elut Plexa PCX, 500 mg/6 mL), which were conditioned with 5 mL of methanol and 5 mL of methanolic sulphuric acid solution. Then, the cartridge was loaded with 25 mL of the sample extract, washed with 8 mL of methanol and dried under vacuum (using a vacuum manifold) for 5-10 minutes. PAs were eluted from the cartridge in two steps with 5 mL each of methanol containing 2.5 % NH_3. The combined eluates were dried under a nitrogen stream in a heated water bath (50 °C, TurboVap) and the drying residue was reconstituted in 1 mL of methanol/water (5/95, v/v). The reconstituted sample extract was filtered through a 0.2 µm centrifuge filters before LC-MS/MS analysis.

The herbal food supplements which were labelled to be prepared as tea infusions were analysed according to the sample preparation procedure of (herbal) tea described in Section 5.2.1.

6. LC-MS/MS ANALYSIS

It should be remarked that RIKILT and BfR use different methods for the LC-MS/MS analysis of PAs. This is due to the fact that these methods were developed and validated independently from each other before the start of this project. Both methods, as developed by RIKILT (Hoogenboom et al., 2011) and by BfR (Bodi et al., 2014) have proven track records and have been in use for several years. The most important difference between the two methods is the use of alkaline chromatographic conditions in the RIKILT method and the use of acidic chromatographic conditions in the BfR method. As a consequence, a different separation profile of the PAs is observed with both methods. Some compounds that are separated under alkaline conditions may not be separated under acidic conditions and vice versa. For this reason no preferred method can be identified, and both can be considered state-of-the-art with respect to sensitivity (lowest reported LODs and LOQs) and number of PAs determined.

For the analysis of the animal-derived products the RIKILT method has been used and for the analysis of the plant-derived products the BfR method has been used (refer to Table 1).

6.1. Animal-derived food products

PAs analysis in the animal-derived food products was performed on a LC-MS/MS system consisting of a Waters Acquity UPLC coupled to a Xevo TQ-S tandem mass spectrometer (Waters, Milford, MA, USA).

Chromatographic separation was achieved on a 150 x 2.1 mm, 1.7 µm particle size, Waters UPLC BEH C18 analytical column (Waters, Milford, MA, USA). Eluent A was prepared from 100 % water containing 6.5 mM ammonium hydroxide and eluent B from 100 % acetonitrile containing 1.2 mM ammonium hydroxide. A gradient elution was performed as follows: 0-1 minutes 100 % A/ 0 % B, 12.0 minutes 50 % A/ 50 % B, 12.2-15 minutes 100 % A/ 0 % B. A flow rate of 400 µL/minute was applied and 5 µL was injected. The column temperature was maintained at 50 °C.

Some isomeric PAs were not baseline separated under the chromatographic conditions used and it was decided to include only one of the isomers in the method. In this way lycopsamine was included in the standard mixture, but its isomers intermedine and indicine were excluded. Similarly, lycopsamine-*N*-oxide was included but its isomer intermedine-*N*-oxide was not. This means that, in case of a positive finding of lycopsamine, it could be that intermedine or a mixture of lycopsamine and intermedine is present in the sample. And in case of lycopsamine-*N*-oxide this could be (a mixture of lycopsamine-*N*-oxide and) intermedine-*N*-oxide. In total 35 PAs were included in the standard mix (representing a method scope of 38 different PAs).

PAs were analysed in positive electrospray ionization mode (ESI+). Two multiple reaction monitoring (MRM) transitions were measured per analyte (Table 10).

Table 10: MS/MS parameters and retention times (RT) for the PAs analysed in animal-derived products by LC-MS/MS in MRM ESI+ mode

Pyrrolizidine alkaloid	Abbr.	Precursor mass (*m/z*)	Fragment mass 1; 2 (*m/z*)	Collision energy fragment 1; 2 (eV)	RT (minutes)
Echimidine	Em	398.2	120.0; 220.0	25; 20	9.59
Echimidine-*N*-oxide	EmNO	414.2	254.0; 352.0	30; 25	7.01
Erucifoline	Er	350.2	94.0; 138.0	40; 30	7.03
Erucifoline-*N*-oxide	ErNO	366.2	94.0; 118.0	40; 30	4.37
Europine	Eu	330.2	94.0; 138.0	35; 25	6.45
Europine-*N*-oxide	EuNO	346.2	172.0; 256.0	30; 25	4.55
Florosenine	Fs	424.2	122.0; 168.0	35; 30	7.83
Heliotrine	He	314.2	138.0; 156.0	30; 25	8.02
Heliotrine-*N*-oxide	HeNO	330.2	111.0; 172.0	35; 25	5.69
Indicine-*N*-oxide	IdNO	316.2	94.0; 172.0	40; 30	4.55
Integerrimine	Ir	336.2	94.0; 120.0	40; 30	9.12
Integerrimine-*N*-oxide	IrNO	352.2	94.0; 136.0	40; 30	6.37
Jacobine	Jb	352.2	94.0; 155.0	40; 30	7.34
epi-Jacobine (IS) [a]	eJb	352.2	94.0; 155.0	40; 30	8.21
Jacobine-*N*-oxide	JbNO	368.2	119.0; 296.0	30; 25	5.02
Jacoline	Jl	370.2	94.0; 138.0	40; 30	5.63
Lasiocarpine	Lc	412.2	120.0; 220.0	30; 20	10.75
Lasiocarpine-*N*-oxide	LcNO	428.2	138.0; 254.0	30; 30	7.64
Lycopsamine [b]	Ly	300.2	94.0; 156.0	35; 30	6.07
Lycopsamine-*N*-oxide [c]	LyNO	316.2	94.0; 172.0	40; 30	4.48
Monocrotaline	Mc	326.2	94.0; 121.0	35; 30	5.88
Monocrotaline-*N*-oxide	McNO	342.2	94.0; 137.0	40; 30	3.84
Otosenine	Ot	382.2	122.0; 168.0	30; 25	5.11

Pyrrolizidine alkaloid	Abbr.	Precursor mass (*m/z*)	Fragment mass 1; 2 (*m/z*)	Collision energy fragment 1; 2 (eV)	RT (minutes)
Retrorsine	Re	352.2	94.0; 138.0	40; 30	7.97
Retrorsine-*N*-oxide	ReNO	368.2	94.0; 118.0	40; 30	5.55
Riddelliine	Rd	350.2	94.0; 138.0	40; 30	7.39
Riddelliine-*N*-oxide	RdNO	366.2	94.0; 118.0	40; 30	5.02
Senecionine	Sn	336.2	94.0; 120.0	40; 30	9.32
Senecionine-*N*-oxide	SnNO	352.2	94.0; 136.0	40; 30	6.50
Seneciphylline	Sp	334.2	94.0; 138.0	40; 30	8.59
Seneciphylline-*N*-oxide	SpNO	350.2	94.0; 118.0	40; 30	5.89
Senecivernine	Sv	336.2	94.0; 120.0	40; 30	9.49
Senecivernine-*N*-oxide	SvNO	352.2	94.0; 136.0	40; 30	6.55
Senkirkine	Sk	366.2	122.0; 168.0	30; 25	6.79
Trichodesmine	Td	354.2	120.0; 222.0	35; 30	8.13
Trichodesmine-*N*-oxide	TdNO	370.2	137.0; 238.0	40; 30	5.55

(a): *epi*-Jacobine (eJb) is a synthetic epimer of jacobine (Jb), that so far has not been reported to occur in nature.
(b): Intermedine (Im) and indicine (Id) coelute with lycopsamine (Ly) under the chromatographic conditions used.
(c): Intermedine-*N*-oxide (ImNO) coelutes with lycopsamine-*N*-oxide (LyNO) under the chromatographic conditions used.

6.2. Plant-derived food products

PAs analysis in the plant-derived food products was performed on a LC-MS/MS system consisting of an UHPLC (Ultimate 3000, Thermo Scientific, San Jose, CA, USA) coupled to a Triple Stage Quadrupole mass spectrometer (TSQ Vantage, Thermo Scientific, San Jose, CA, USA).

Chromatographic separation was achieved on a 150 x 2.1 mm, 1.9 µm particle size, C18 Hypersil Gold column fitted with a guard column (Thermo Scientific, Germany). Eluent A was prepared from 100 % water containing 0.1 % formic acid and 5 mM ammonium formate and eluent B from 95 % methanol and 5 % water containing 0.1 % formic acid and 5 mM ammonium formate. A gradient elution was performed as follows: 0-0.5 minutes 95 % A/ 5 % B, 7.0 minutes 50 % A/ 50 % B, 7.5 minutes 20 % A/ 80 % B, 7.6-9.0 minutes 0 % A/ 100 % B, 9.1-15 minutes 95 % A/ 5 % B. A flow rate of 300 µL/minute was applied and 10 µL was injected. The column temperature was maintained at 40 °C. Some isomeric PAs were not baseline separated and it was decided to include only one of the isomers in the PA standard mix. As indicine coelutes with its isomer intermedine only intermedine was included. Similarly, intermedine-*N*-oxide was included but its isomer indicine-*N*-oxide was not. This means that, in case of a positive finding of intermedine, it could be that indicine or a mixture of intermedine and indicine is present in the sample. And in case of intermedine-*N*-oxide this could be (a mixture of intermedine-*N*-oxide and) indicine-*N*-oxide. In total 28 PAs were included in the standard mix (representing a method scope for 30 different PAs).

PAs were analysed in positive electrospray ionization mode (ESI+). Two MRM transitions were measured per analyte (Table 11).

Table 11: MS/MS parameters and retention times (RT) for PAs analysed in tea infusion by LC-MS/MS in MRM ESI+ mode

Pyrrolizidine alkaloid	Abbr.	Precursor mass (*m/z*)	Fragment mass 1; 2 (*m/z*)	Collision energy fragment 1; 2 (eV)	RT (minutes)
Echimidine	Em	398.2	120.3; 220.3	23; 17	8.10
Echimidine-*N*-oxide	EmNO	414.2	254.1; 352.1	32; 27	8.09
Erucifoline	Er	350.2	120.3; 138.1	32; 30	4.87

Pyrrolizidine alkaloid	Abbr.	Precursor mass (*m/z*)	Fragment mass 1; 2 (*m/z*)	Collision energy fragment 1; 2 (eV)	RT (minutes)
Erucifoline-*N*-oxide	ErNO	366.1	136.1; 120.1	30; 33	5.29
Europine	Eu	330.1	138.1; 156.2	20; 28	5.35
Europine-*N*-oxide	EuNO	346.1	111.2; 172.1	41; 31	5.75
Heliotrine	He	314.2	138.3; 156.3	19; 28	6.84
Heliotrine-*N*-oxide	HeNO	330.2	138.2; 172.1	22; 27	7.15
Intermedine [(a)]	Im	300.1	138.3; 156.3	18; 28	5.55
Intermedine-*N*-oxide [(b)]	ImNO	316.1	111.2; 138.1	37; 26	6.04
Jacobine	Jb	352.1	120.1; 155.2	36; 29	5.38
Jacobine-*N*-oxide	JbNO	368.1	120.1; 296.1	32; 23	5.63
Lasiocarpine	Lc	412.2	120.2; 336.3	30; 17	9.05
Lasiocarpine-*N*-oxide	LcNO	428.2	136.1; 254.1	29; 27	9.31
Lycopsamine	Ly	300.1	138.3; 156.3	18; 28	5.66
Lycopsamine-*N*-oxide	LyNO	316.1	111.2; 138.1	37; 26	6.15
Monocrotaline	Mc	326.2	120.3; 237.3	35; 25	4.87
Monocrotaline-*N*-oxide	McNO	342.1	118.3; 137.4	37; 29	5.12
Retrorsine	Re	352.2	120.3; 138.3	27; 29	6.43
Retrorsine-*N*-oxide	ReNO	368.2	136.2; 118.2	30; 40	6.52
Senecionine	Sn	336.2	120.2; 138.2	27; 29	7.44
Senecionine-*N*-oxide	SnNO	352.2	118.1; 136.3	28; 27	7.54
Seneciphylline	Sp	334.2	120.3; 138.4	26; 28	6.67
Seneciphylline-*N*-oxide	SpNO	350.2	118.2; 136.3	36; 32	6.89
Senecivernine	Sv	336.2	120.1; 138.1	27; 27	7.36
Senecivernine-*N*-oxide	SvNO	352.1	118.1; 120.1	30; 36	7.63
Senkirkine	Sk	366.2	150.3; 168.2	24; 28	8.28
Trichodesmine	Td	354.2	120.3; 222.3	35; 28	6.49

(a): Indicine (Id) coelutes with intermedine (Im) under the chromatographic conditions used.
(b): Indicine-*N*-oxide (IdNO) coelutes with intermedine-*N*-oxide (ImNO) under the chromatographic conditions used.

7. QUALITY CONTROL

Various quality control measures were implemented and several quality control (QC) criteria fulfilled for the determination of PAs in both animal- and plant-derived products. The QC criteria, which had been defined during the in-house validation differed depending on the method used and the standard taken as reference, which is the SANCO/12571/2013 guideline (SANCO, 2013).

In order to guarantee the performance of the LC-MS/MS system a quality control standard (PA standard mix of 10 ng/mL in solvent) was injected at the beginning of each series of measurements. The intensity of three PA transitions was monitored (one in each of the three MRM windows) and had to meet QC-criteria before a LC-MS/MS analysis was started. QC criteria were defined as follows:

- In case of animal-derived products, whose analyses are specially demanding as far as sensitivity is concerned, the analyte signal (area) of the transition of the weakest ion in each MRM window of the quality control standard should have a signal to noise ratio of at least 100 to insure sufficient sensitivity of the system.

- In case of plant-derived products, the performance of the LC-MS/MS system was checked by injecting a 1 ng/mL PA standard mix in solvent. Peak areas and retention times were recorded in a quality control chart. The relative retention times of each quality control-standard injection has to be below the maximum permitted deviation of 2.5 % with regard to the mean

value in the control chart, and the analyte signal (area) has to be below the maximum permitted deviation of 25 % with regard to the mean value in the control chart.

In order to guarantee sufficient performance during sample preparation (extraction, SPE) each sequence of samples included recovery samples (i.e. blank samples that are analysed in the same way as the other samples and that are spiked with known amounts of PA standards). For all the samples analysed, the recovery of PAs should preferably be within 70 and 120 %. Because matrix-matched calibration is used, a lower recovery may be acceptable provided that the sensitivity of the measurement is not impaired.

In addition, for the analysis of animal-derived food products:

- An internal standard (*epi*-jacobine) was added to all samples before extraction. This internal standard was not used to correct for variations in recovery, but as a general quality control indicator. When the area of the internal standard in a particular sample extract fell below a critical threshold value (50 % compared to the average area in the MMS extracts), sufficient sensitivity of measurement could not be guaranteed and the sample was reprocessed.

- The observed retention time and ion ratio for an individual PA should fall within a critical range, calculated from the MMS samples that are injected at the beginning and at the end of each series of measurements. Retention times should be within 0.2 minutes from the average value of the retention time in the MMS samples and the ion ratio should fall within 30 % from the average value of the ion ratio in the MMS samples as stated in SANCO/12571/2013 (SANCO, 2013)

- Stability of samples stored at -20 °C was assessed halfway and at the end of the project by measurement of a set of samples (milk, egg, beef meat, pork meat, poultry meat) stored at -20 °C and at -80 °C. The stability samples were prepared at the beginning of the project by spiking fresh blank materials (see Section 4.2).

In addition, for the analysis of plant-derived food products:

- In order to reduce the uncertainty of measurement each sample was analysed by duplicate injection and calibration. The deviation between both results had to be below the maximum permitted deviation of 35 % with regard to the mean value in the respective analyte control chart.

RESULTS

8. METHOD VALIDATION

8.1. Animal-derived food products

The determination of PAs in animal-derived products was in-house validated with respect to the limit of quantification (LOQ), recovery, accuracy and linearity. In the absence of a specific regulation or guidance document for the validation of a method for the determination of plant toxins in products of animal origin, the Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed SANCO/12751/2013 (SANCO, 2013) was used as guidance document for the method validation and on-going analytical quality control.

Validation was performed by spiking three different samples of milk and of eggs, two different samples of beef and of pork meat and one sample of poultry meat. The spiking was done in six-fold at three different levels (0.1, 0.5 and 2.5 µg/L in milk, 0.5, 2.5 and 10 µg/kg in eggs, and 0.25, 1 and 5 µg/kg in meat). Linearity over the working range was assessed through the incorporation of 7 matrix-matched calibration standards (0-5 µg/L for milk, 0-10 µg/kg for eggs, and 0-10 µg/kg for meat).

Recovery was determined by including three blank samples in each validation experiment, of which the final extracts were spiked with the corresponding amount of PA standards (at 2.5 µg/L in milk, 10 µg/kg in eggs, and 5 µg/kg in meat).

The method was not specifically validated for cheese and for liver because of the relatively small number of items collected and the variability expected within the collected samples (soft versus hard cheese, liver of beef vs pork vs poultry). It was therefore decided to use the validated procedures for milk and for liver, respectively. The quality of the analysis was assured by analysing in duplicate each individual sample, of which one was fortified with a multi-PA mix at 10 µg/kg.

8.1.1. Limit of quantification (LOQ) and limit of detection (LOD)

Regarding the methods developed for milk, eggs and meat, sufficiently low LOQs for the individual PAs were obtained to allow determination at the lowest required performance level (0.1 µg/L in milk, 0.5 µg/L in eggs and 0.25 µg/kg in meat). For yoghurt it was found that a similar performance as for milk could be achieved, for cheese the performance was similar as for eggs, and for liver the performance was comparable to that of beef meat. Only in some specific cases, e.g. beef meat, it was observed that due to strong matrix suppression or due to co-eluting matrix interferences, the lowest spiking level of 0.25 µg/kg was not in all cases feasible for the PAs, most notably for monocrotaline-*N*-oxide. An overview of the LOD and LOQ values established for the different matrices is given in Table 12. For the major matrices (milk, eggs, meat) LOQs had been determined during the initial validation and were set equal to the lowest spiking level for which acceptable accuracy data had been obtained and which was equal to the lowest spiking level included in the MMS. LODs for these matrices were established on the basis of the average performance observed during the analysis of the various sample series. The requirement for the LOD values was that both product-to-ion transitions were observed with a S/N ratio of at least 6. The LOD and LOQ values for the minor matrices (yoghurt, cheese, liver) were determined on the basis of the performance observed during the analysis of the various sample series. LOQs for the minor matrices were set equal to the lowest spiking level included in the MMS. For the LODs the requirement was that both product-to-ion transitions were observed with a S/N ratio of at least 6.

Table 12: Established LOD and LOQ values for PAs in animal-derived products[(a)]

Pyrrolizidine alkaloid	Abbr.	Milk, yoghurt		Egg, cheese		Pork, poultry meat		Beef meat, liver	
		LOD (µg/L)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)
Echimidine	Em	0.04	0.10	0.10	0.25	0.10	0.25	0.10	0.25
Echmidine-*N*-oxide	EmNO	0.05	0.10	0.15	0.25	0.15	0.25	0.15	0.25
Erucifoline	Er	0.05	0.10	0.15	0.25	0.15	0.25	0.15	0.25
Erucifoline-*N*-oxide	ErNO	0.05	0.10	0.15	0.25	0.15	0.25	0.15	0.25
Europine	Eu	0.03	0.10	0.10	0.25	0.10	0.25	0.10	0.25
Europine-*N*-oxide	EuNO	0.04	0.10	0.05	0.25	0.05	0.25	0.10	0.25

Pyrrolizidine alkaloid	Abbr.	Milk, yoghurt		Egg, cheese		Pork, poultry meat		Beef meat, liver	
		LOD (µg/L)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)
Florosenine	Fs	0.03	0.10	0.10	0.25	0.10	0.25	0.10	0.25
Heliotrine	He	0.03	0.10	0.05	0.25	0.05	0.25	0.10	0.25
Heliotrine-*N*-oxide	HeNO	0.04	0.10	0.05	0.25	0.05	0.25	0.10	0.25
Indicine-*N*-oxide	IdNO	0.05	0.10	0.05	0.25	0.05	0.25	0.10	0.25
Integerrimine	Ir	0.04	0.10	0.10	0.25	0.05	0.25	0.05	0.25
Integerrimine-*N*-oxide	IrNO	0.05	0.10	0.10	0.25	0.10	0.25	0.15	0.25
Jacobine	Jb	0.05	0.10	0.15	0.25	0.15	0.25	0.15	0.25
Jacobine-*N*-oxide	JbNO	0.05	0.10	0.15	0.25	0.15	0.25	0.15	0.25
Jacoline	Jl	0.04	0.10	0.10	0.25	0.15	0.25	0.15	0.25
Lasiocarpine	Lc	0.03	0.10	0.05	0.25	0.05	0.25	0.05	0.25
Lasiocarpine-*N*-oxide	LcNO	0.04	0.10	0.10	0.25	0.10	0.25	0.15	0.25
Lycopsamine	Ly	0.05	0.10	0.10	0.25	0.10	0.25	0.10	0.25
Lycopsamine-*N*-oxide	LyNO	0.05	0.10	0.05	0.25	0.05	0.25	0.05	0.25
Monocrotaline	Mc	0.05	0.10	0.10	0.25	0.10	0.25	0.15	0.25
Monocrotaline-*N*-oxide	McNO	0.05	0.10	0.10	0.25	0.10	0.25	0.25	1.00 (b)
Otosenine	Ot	0.04	0.10	0.10	0.25	0.10	0.25	0.10	0.25
Retrorsine	Re	0.04	0.10	0.10	0.25	0.10	0.25	0.10	0.25
Retrorsine-*N*-oxide	ReNO	0.05	0.10	0.10	0.25	0.15	0.25	0.15	0.25
Riddelliine	Rd	0.05	0.10	0.15	0.25	0.15	0.25	0.15	0.25
Riddelliine-*N*-oxide	RdNO	0.05	0.10	0.15	0.25	0.15	0.25	0.15	0.25
Senecionine	Sn	0.04	0.10	0.10	0.25	0.05	0.25	0.05	0.25
Senecionine-*N*-oxide	SnNO	0.05	0.10	0.10	0.25	0.10	0.25	0.15	0.25
Senkirkine	Sk	0.04	0.10	0.10	0.25	0.10	0.25	0.10	0.25
Seneciphylline	Sp	0.04	0.10	0.10	0.25	0.10	0.25	0.10	0.25
Seneciphylline-*N*-oxide	SpNO	0.05	0.10	0.10	0.25	0.10	0.25	0.15	0.25
Senecivernine	Sv	0.04	0.10	0.10	0.25	0.05	0.25	0.10	0.25
Senecivernine-*N*-oxide	SvNO	0.05	0.10	0.10	0.25	0.10	0.25	0.15	0.25
Trichodesmine	Td	0.05	0.10	0.10	0.25	0.10	0.25	0.10	0.25
Trichodesmine-*N*-oxide	TdNO	0.05	0.10	0.10	0.25	0.10	0.25	0.15	0.25

(a): Limits of quantification (LOQs) for milk, egg, beef meat, pork meat and poultry meat were established during the in-house validation of the method and were set equal to the lowest spiking level included in the MMS. LOQs for yoghurt, cheese and liver were determined during the analysis of the sample series and were set equal to the lowest spiking level included in the MMS. Limits of detection (LODs) for all matrices were established during the analysis of the sample series. LODs were the level at which both precursor to product transitions could be detected with a S/N of 6.

(b): higher LOQ due to matrix interference.

8.1.2. Recovery and accuracy

Recovery was determined at one level (2.5 µg/L in milk, 10 µg/kg in eggs and at 5 µg/kg in meat). Accuracy was determined at three levels (low, medium, high) in each matrix. The recovery and accuracy results for milk, eggs and meat are shown in Table 13, Table 14 and Table 15, respectively.

Table 13: Average recovery (n = 3) of individual PAs and accuracy in milk

Pyrrolizidine alkaloid	Abbr.	Recovery (%) 2.5 µg/L	RSD recovery (%) 2.5 µg/L	Accuracy (%) 0.1 µg/L	Accuracy (%) 0.5 µg/L	Accuracy (%) 2.5 µg/L
Echimidine	Em	103	31 (b)	91	100	99
Echmidine-*N*-oxide	EmNO	98	9	98	101	99
Erucifoline	Er	93	7	86	104	107
Erucifoline-*N*-oxide	ErNO	100	21	106	122	112
Europine	Eu	100	13	92	98	97
Europine-*N*-oxide	EuNO	91	9	111	112	106
Florosenine	Fs	102	10	87	87	95
Heliotrine	He	95	18	95	101	99
Heliotrine-*N*-oxide	HeNO	96	3	97	107	102
Indicine-*N*-oxide	IdNO	86	15	114	113	103
Integerrimine	Ir	96	19	87	100	98
Integerrimine-*N*-oxide	IrNO	107	19	102	101	96
Jacobine	Jb	95	5	96	104	102
Jacobine-*N*-oxide	JbNO	86	4	108	115	110
Jacoline	Jl	97	12	91	96	100
Lasiocarpine	Lc	101	12	90	110	111
Lasiocarpine-*N*-oxide	LcNO	93	11	98	103	98
Lycopsamine	Ly	97	14	103	105	103
Lycopsamine-*N*-oxide	LyNO	94	8	87	98	93
Monocrotaline	Mc	93	16	100	104	94
Monocrotaline-*N*-oxide	McNO	90	11	90	100	97
Otosenine	Ot	95	6	98	108	107
Retrorsine	Re	92	25	105	99	96
Retrorsine-*N*-oxide	ReNO	92	6	110	108	100
Riddelliine	Rd	81	22	91	90	83
Riddelliine-*N*-oxide	RdNO	45 (a)	12	66	75	65
Senecionine	Sn	96	31 (b)	88	103	101
Senecionine-*N*-oxide	SnNO	95	1	105	101	99
Senkirkine	Sk	92	8	89	104	101
Seneciphylline	Sp	89	17	89	95	95
Seneciphylline-*N*-oxide	SpNO	74	19	83	85	68
Trichodesmine	Td	97	27 (b)	98	97	99
Trichodesmine-*N*-oxide	TdNO	89	9	102	105	96

(a): Recovery falls outside the desired range of 70-120 %.
(b): Relative Standard Deviation (RSD) is larger than the desired maximum of 25 %.

Table 14: Average recovery (n = 3) of individual PAs and accuracy in eggs

Pyrrolizidine alkaloid	Abbr.	Recovery (%) 10 µg/kg	RSD recovery (%) 10 µg/kg	Accuracy (%) 0.5 µg/kg	Accuracy (%) 2.5 µg/kg	Accuracy (%) 10 µg/kg
Echimidine	Em	92	12	95	92	92
Echmidine-*N*-oxide	EmNO	86	3	94	100	98
Erucifoline	Er	85	11	97	99	100
Erucifoline-*N*-oxide	ErNO	77	7	97	100	100
Europine	Eu	88	11	94	100	101
Europine-*N*-oxide	EuNO	96	10	99	102	101
Florosenine	Fs	89	7	95	99	99
Heliotrine	He	93	11	95	99	99
Heliotrine-*N*-oxide	HeNO	95	9	95	100	102
Indicine-*N*-oxide	IdNO	92	11	98	101	101
Integerrimine	Ir	79	17	100	98	88
Integerrimine-*N*-oxide	IrNO	93	6	97	100	100
Jacobine	Jb	83	9	94	98	98
Jacobine-*N*-oxide	JbNO	94	5	94	100	101
Jacoline	Jl	84	12	94	101	106
Lasiocarpine	Lc	90	15	81	88	84
Lasiocarpine-*N*-oxide	LcNO	91	6	97	99	98
Lycopsamine	Ly	81	16	99	104	105
Lycopsamine-*N*-oxide	LyNO	100	7	98	102	100
Monocrotaline	Mc	86	11	96	103	107
Monocrotaline-*N*-oxide	McNO	73	10	87	100	100
Otosenine	Ot	97	13	95	101	102
Retrorsine	Re	89	11	94	100	101
Retrorsine-*N*-oxide	ReNO	92	9	97	100	102
Riddelliine	Rd	61 [(a)]	11	100	98	92
Riddelliine-*N*-oxide	RdNO	30 [(a)]	20	101	102	90
Senecionine	Sn	103	33 [(b)]	85	91	94
Senecionine-*N*-oxide	SnNO	96	7	95	102	101
Senkirkine	Sk	94	8	95	102	101
Seneciphylline	Sp	72	7	89	92	98
Seneciphylline-*N*-oxide	SpNO	56 [(a)]	19	102	99	93
Trichodesmine	Td	85	10	96	100	98
Trichodesmine-*N*-oxide	TdNO	91	6	94	101	101

(a): Recovery falls outside the desired range of 70-120 %.
(b): Relative Standard Deviation (RSD) is larger than the desired maximum of 25 %.

Table 15: Average recovery (n = 5) of individual PAs and accuracy in meat (beef, pork and poultry)

Pyrrolizidine alkaloid	Abbr.	Recovery (%) 5 µg/kg	RSD recovery (%) 5 µg/kg	Accuracy (%) 0.25 µg/kg	Accuracy (%) 1 µg/kg	Accuracy (%) 5 µg/kg
Echimidine	Em	78	8	112	101	99
Echmidine-*N*-oxide	EmNO	85	17	125	106	94
Erucifoline	Er	81	10	97	95	95
Erucifoline-*N*-oxide	ErNO	77	22	118	103	91
Europine	Eu	81	9	102	98	96
Europine-*N*-oxide	EuNO	88	13	110	106	99
Florosenine	Fs	80	9	110	102	95
Heliotrine	He	79	9	106	98	97
Heliotrine-*N*-oxide	HeNO	91	17	111	106	98
Indicine-*N*-oxide	IdNO	90	17	113	107	98
Integerrimine	Ir	82	12	99	98	98
Integerrimine-*N*-oxide	IrNO	86	19	114	109	94
Jacobine	Jb	80	11	101	94	93
Jacobine-*N*-oxide	JbNO	83	16	120	104	94
Jacoline	Jl	81	8	97	97	95
Lasiocarpine	Lc	81	11	98	104	107
Lasiocarpine-*N*-oxide	LcNO	87	24	138	111	95
Lycopsamine	Ly	81	15	94	93	97
Lycopsamine-*N*-oxide	LyNO	90	15	114	105	97
Monocrotaline	Mc	83	9	99	97	95
Monocrotaline-*N*-oxide	McNO	84	30 [(b)]	114 [(c)]	99 [(c)]	93 [(c)]
Otosenine	Ot	82	7	112	102	96
Retrorsine	Re	80	12	95	94	95
Retrorsine-*N*-oxide	ReNO	86	19	127	109	94
Riddelliine	Rd	65 [(a)]	8	93	90	88
Riddelliine-*N*-oxide	RdNO	41 [(a)]	10	102	88	81
Senecionine	Sn	80	13	112	101	99
Senecionine-*N*-oxide	SnNO	86	19	137	112	96
Senkirkine	Sk	79	5	109	99	94
Seneciphylline	Sp	74	10	97	95	94
Seneciphylline-*N*-oxide	SpNO	63 [(a)]	15	118	100	88
Trichodesmine	Td	82	12	103	98	97
Trichodesmine-*N*-oxide	TdNO	81	19	125	108	93

(a): Recovery falls outside the desired range of 70-120 %.
(b): Relative Standard Deviation (RSD) is larger than the desired maximum of 25 %.
(c): Average of three validation days, excluding beef meat, due to severe matrix interference.

From Tables 13 and 14 it can be seen that for most PAs good recoveries were obtained for milk (average recovery: 92 %) and eggs (average recovery: 85 %). Table 15 shows that slightly lower recoveries were obtained for meat (average recovery: 80 %). However, differences between individual PAs can be observed. Riddelliine-*N*-oxide appeared to be the most critical compound, producing recoveries of only 30-45 %. This is a rather polar compound and might not be well retained on the SPE cartridge. The pH of the washing solutions used during SPE clean-up could be another factor of importance. Some PAs such as riddelliine-*N*-oxide are particularly sensitive to elevated pH and may degrade relatively easily. Care should be taken that during sample clean-up a pH of 10 is not exceeded to prevent losses of sensitive PAs due to degradation.

Accuracies obtained for the PAs in milk, eggs and meat were generally well within the preferred range of 70-120 %, in many cases within 80-110 %. Somewhat less favourable results were obtained for the pyrrolizidine alkaloid *N*-oxides (PANOs) at the lowest level in meat: due to stronger matrix effects (suppression) peak intensities were in most cases rather low, especially for beef meat, increasing the error in the measurements.

8.1.3. Linearity, specificity and matrix effects

Linearity over the intended working range (0-5 µg/L for milk, 0-10 µg/L for eggs and 0-10 µg/kg for meat) was assessed using a set of 7 matrix-matched calibration samples. Linearity for individual PAs varied from 0.994 to 1.000 in milk, from 0.989 to 1.000 in eggs, from 0.991 to 1.000 in poultry meat, from 0.998 to 1.000 in pork meat and from 0.993 to 1.000 in beef meat. Acceptable linearity was thus obtained for all matrices.

Specificity was in general very good for milk and eggs, with few interfering matrix components for the individual PAs. Figure 1 shows an LC-MS/MS chromatogram (MRM mode) of a blank milk sample and Figure 2 a blank milk sample spiked with a mixture of PAs at 0.5 µg/L. The MRM chromatograms are practically free of matrix interferences, and sensitivity is good, enabling low LODs. For eggs, similar results as for milk were obtained (data not shown). Blank egg matrix in general did not contain significant interfering compounds.

However, a different situation was observed for meat. Figure 3 shows an LC-MS/MS MRM chromatogram of a blank pork muscle tissue sample, and Figure 4 a sample spiked at 0.5 µg/kg PAs. A large number of endogenous components can be seen in the chromatograms of the blank sample. Many of these components did not interfere with the PAs of interest (eluting at different retention times than the compounds of interest), but it is evident that muscle tissue is a more challenging matrix than eggs or milk. In beef meat, a strong interfering matrix peak prevented the determination of monocrotaline-*N*-oxide. Matrix suppression was also more significant in muscle tissue than in milk or eggs, particularly for the PANOs. Matrix suppression for individual compounds appeared to be different between the three meat matrices. Therefore it was decided to use meat-specific matrix-matched calibration and QC samples for poultry, pork and beef meat analysis.

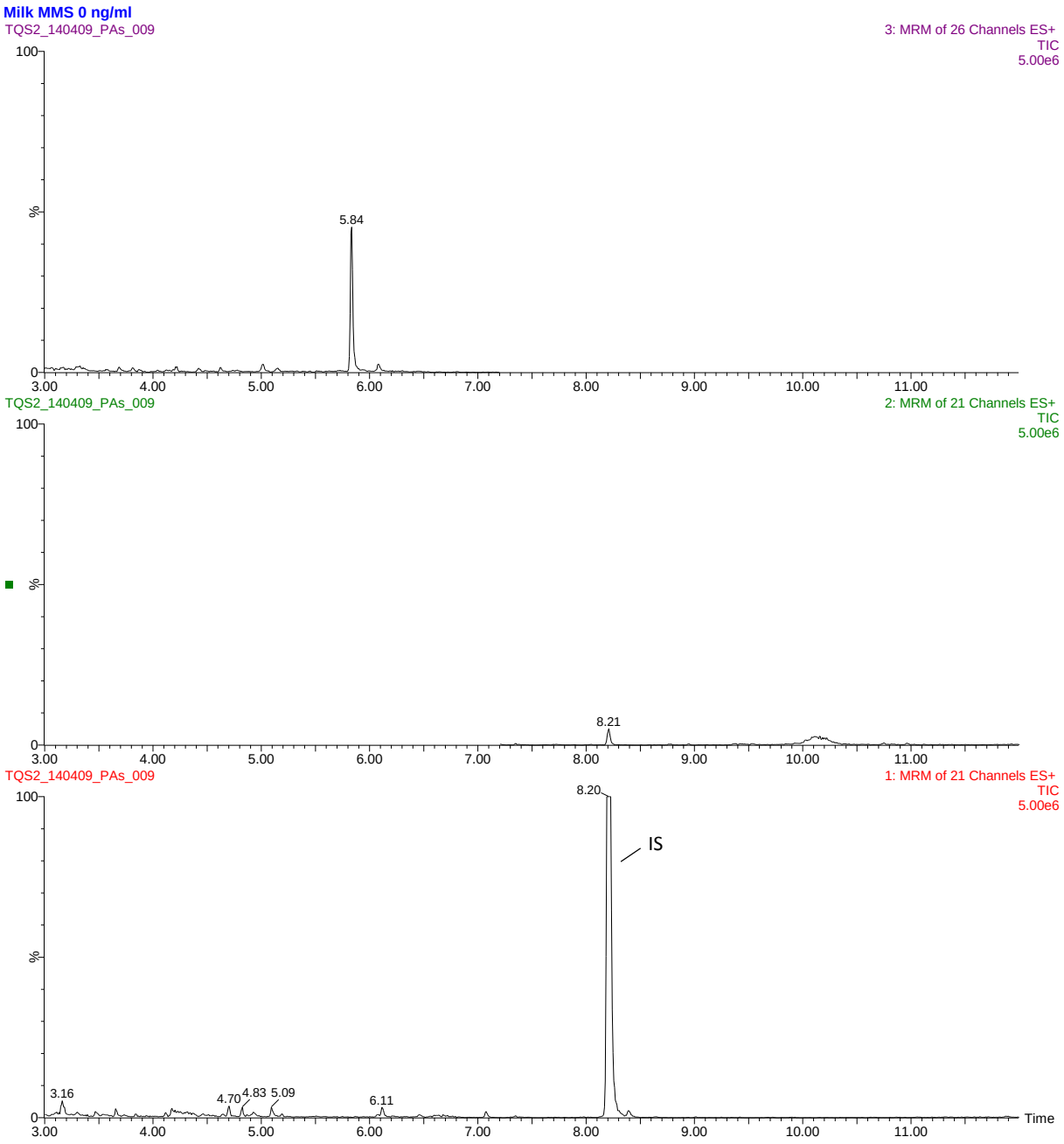

Figure 1: LC-MS/MS MRM chromatograms of a blank milk sample fortified with the internal standard (IS, 10 µg/L)

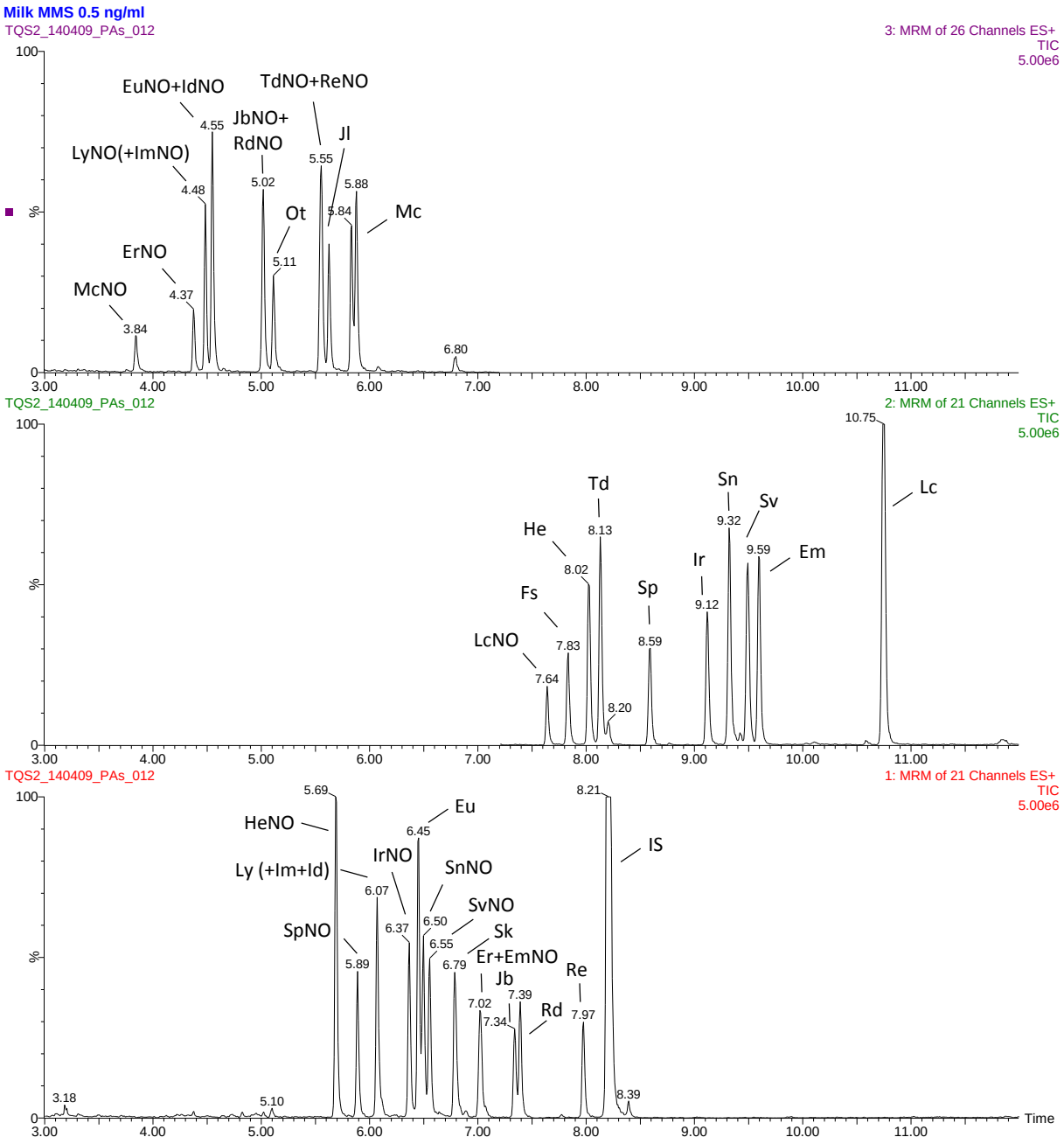

Figure 2: LC-MS/MS MRM chromatograms of a blank milk sample fortified with 0.5 µg/L PA standards and the internal standard (IS, 10 µg/L). Abbreviations are explained in Table 10.

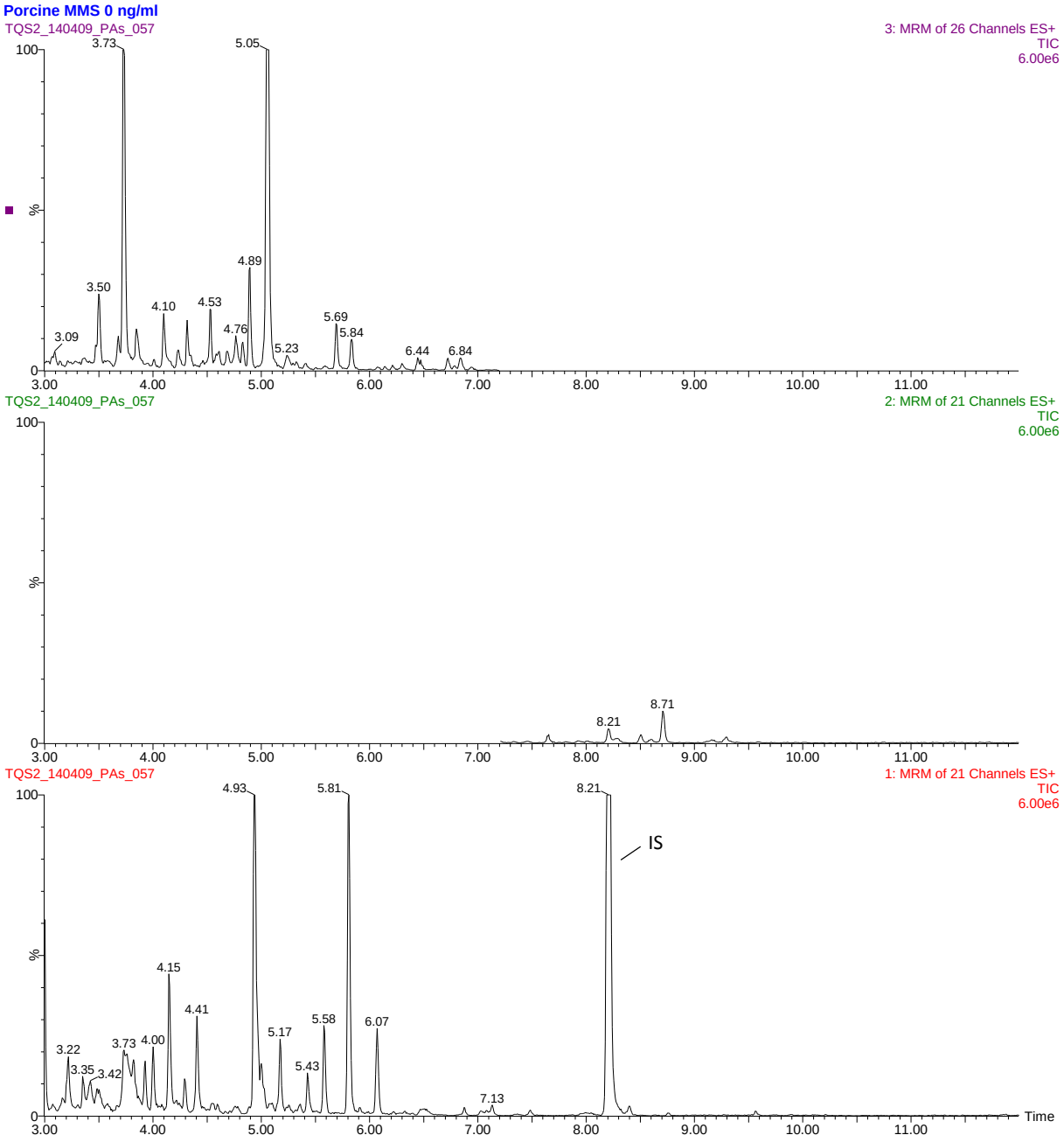

Figure 3: LC-MS/MS MRM chromatograms of a blank pork meat sample fortified with the internal standard (IS, 10 µg/kg)

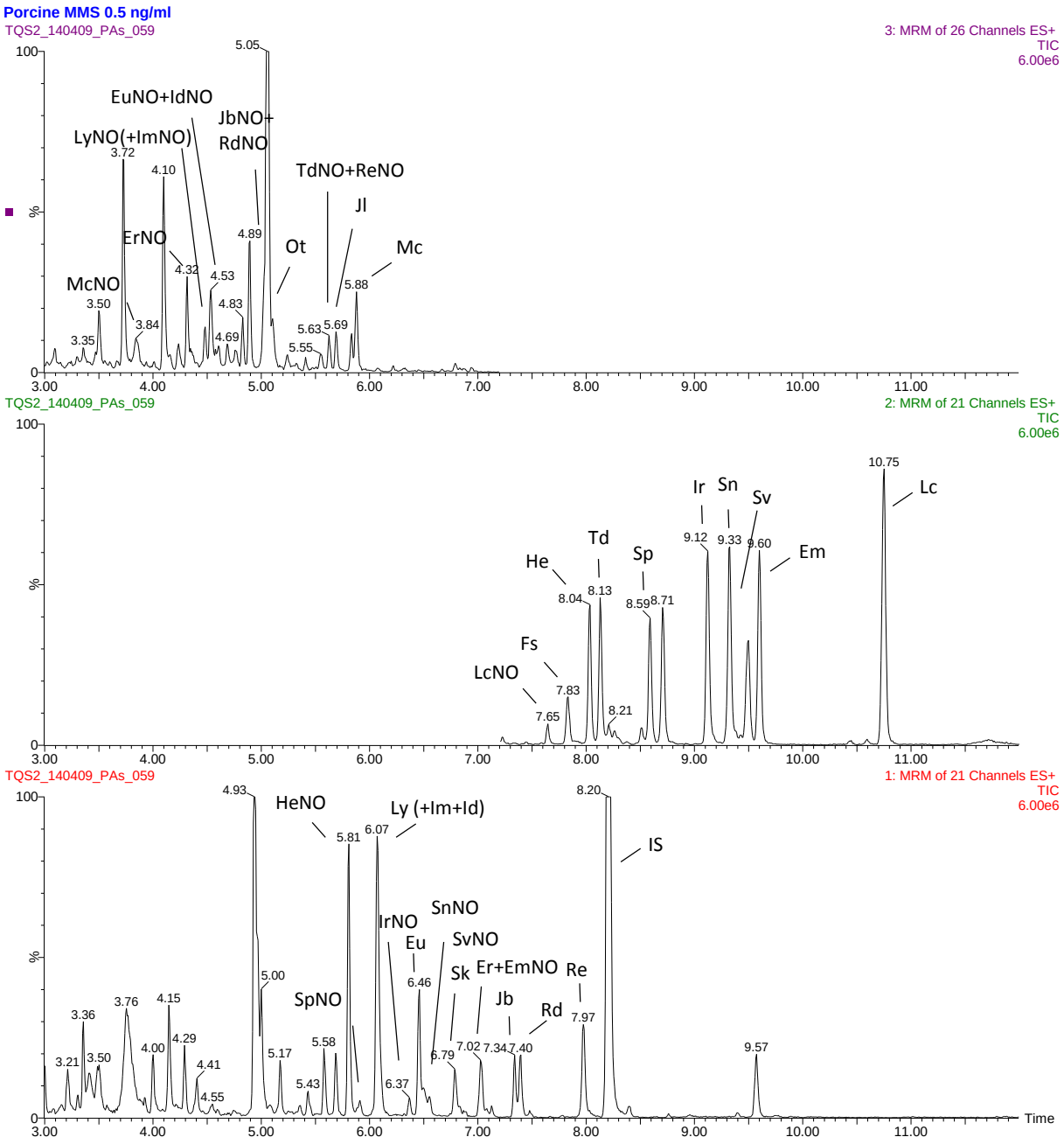

Figure 4: LC-MS/MS MRM chromatograms of a blank pork meat sample fortified with 0.5 µg/L PA standards and the internal standard (IS, 10 µg/kg). Abbreviations are explained in Table 10.

8.2. Plant-derived food products

The determination of PAs in (herbal) teas and food supplements was validated in-house with respect to the LOD, LOQ, recovery and linearity.

8.2.1. Limit of quantification (LOQ) and limit of detection (LOD)

Table 16 and Table 17 show the individual LOD and LOQ values obtained for plant-derived products, which were established according to the standard method DIN 32645 (DIN, 1986). For this purpose three replicates of five calibration levels that cover the lower calibration range were prepared and analysed.

The LOD values obtained for (herbal) tea were in the range of 0.007-0.027 µg/L (corresponding to 0.5-2.0 µg/kg dry tea), whereas the LOQ values were in the range of 0.023-0.085 µg/L (corresponding to 1.7-6.4 µg/kg dry tea). These values indicate that the proposed method is suitable for the detection of PAs and the LOQs are considered to be sufficiently low for analysis of tea infusions.

Table 16: LOD and LOQ values obtained during the in-house validation of the analytical method for the determination of PAs in (herbal) tea infusion (µg/L), and also expressed as dry tea (µg/kg) (n = 3)

Pyrrolizidine alkaloid	Abbr.	(Herbal) tea infusion		Dry (herbal) tea	
		LOD (µg/L)	LOQ (µg/L)	LOD (µg/kg)	LOQ (µg/kg)
Echimidine	Em	0.011	0.035	0.8	2.6
Echimidine-*N*-oxide	EmNO	0.025	0.081	1.9	6.1
Erucifoline	Er	0.008	0.025	0.6	1.9
Erucifoline-*N*-oxide	ErNO	0.016	0.051	1.2	3.8
Europine	Eu	0.009	0.028	0.7	2.1
Europine-*N*-oxide	EuNO	0.009	0.031	0.7	2.3
Heliotrine	He	0.007	0.023	0.5	1.7
Heliotrine-*N*-oxide	HeNO	0.008	0.027	0.6	2.0
Intermedine	Im	0.013	0.041	1.0	3.1
Intermedine-*N*-oxide	ImNO	0.016	0.051	1.2	3.8
Jacobine	Jb	0.017	0.053	1.3	4.0
Jacobine-*N*-oxide	JbNO	0.017	0.056	1.3	4.2
Lasiocarpine	Lc	0.011	0.032	0.8	2.4
Lasiocarpine-*N*-oxide	LcNO	0.012	0.037	0.9	2.8
Lycopsamine	Ly	0.027	0.085	2.0	6.4
Lycopsamine-*N*-oxide	LyNO	0.020	0.065	1.5	4.9
Monocrotaline	Mc	0.012	0.037	0.9	2.8
Monocrotaline-*N*-oxide	McNO	0.023	0.072	1.7	5.4
Retrorsine	Re	0.011	0.036	0.8	2.7
Retrorsine-*N*-oxide	ReNO	0.019	0.061	1.4	4.6
Senecionine	Sn	0.024	0.079	1.8	5.9
Senecionine-*N*-oxide	SnNO	0.012	0.039	0.9	2.9
Seneciphylline	Sp	0.017	0.053	1.3	4.0
Seneciphylline-*N*-oxide	SpNO	0.012	0.036	0.9	2.7
Senecivernine	Sv	0.023	0.071	1.7	5.3
Senecivernine-*N*-oxide	SvNO	0.011	0.035	0.8	2.6
Senkirkine	Sk	0.011	0.032	0.8	2.4
Trichodesmine	Td	0.013	0.041	1.0	3.1

For food supplements, the subgroup of bee products yielded low LOD (0.2-0.6 µg/kg) and LOQ (0.5-2.1 µg/kg) values. The LOD (0.3-2.3 µg/kg) and LOQ (0.9-8.3 µg/kg) values for the dry food supplements were comparable to the values obtained for the herbal teas (expressed as dry tea). The LOD (0.9-3.8 µg/kg) and LOQ (3.3-13.6 µg/kg) values obtained for oil-based supplements were somewhat higher. Overall, the LOQs for the individual PAs were found to be sufficiently low in order to allow determination at a relevant performance level.

Table 17: LOD and LOQ values (µg/kg) obtained during the in-house validation of the analytical method for the determination of PAs in (herbal) food supplements (n = 3)

Pyrrolizidine alkaloid	Abbr.	Dry food supplements		Bee products supplements		Oil-based supplements	
		LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)
Echimidine	Em	0.3	0.9	0.4	1.4	1.8	6.5
Echimidine-*N*-oxide	EmNO	0.5	1.9	0.4	1.5	1.6	5.5
Erucifoline	Er	1.7	5.6	0.5	1.8	2.9	10.3
Erucifoline-*N*-oxide	ErNO	0.6	2.3	0.4	1.4	2.7	9.8
Europine	Eu	0.3	1.1	0.5	1.9	2.0	7.3
Europine-*N*-oxide	EuNO	0.4	1.4	0.4	1.3	1.1	3.8
Heliotrine	He	0.3	1.1	0.2	0.8	2.1	7.7
Heliotrine-*N*-oxide	HeNO	0.4	1.3	0.2	0.8	1.8	6.4
Intermedine	Im	0.5	1.7	0.4	1.6	1.9	6.8
Intermedine-*N*-oxide	ImNO	0.5	1.6	0.2	0.6	1.7	6.2
Jacobine	Jb	2.2	8.0	0.5	1.7	3.1	11.2
Jacobine-*N*-oxide	JbNO	0.5	1.7	0.2	0.5	2.2	7.8
Lasiocarpine	Lc	0.4	1.3	0.2	0.7	1.1	4.1
Lasiocarpine-*N*-oxide	LcNO	0.6	2.3	0.3	1.1	1.4	5.0
Lycopsamine	Ly	0.5	1.7	0.2	0.8	2.2	8.0
Lycopsamine-*N*-oxide	LyNO	0.5	1.9	0.3	1.1	1.7	6.0
Monocrotaline	Mc	0.4	1.4	0.3	0.9	2.1	7.5
Monocrotaline-*N*-oxide	McNO	2.3	8.3	0.5	1.6	3.8	13.6
Retrorsine	Re	2.1	7.4	0.3	1.0	2.6	9.4
Retrorsine-*N*-oxide	ReNO	0.5	1.9	0.4	1.5	1.5	5.5
Senecionine	Sn	0.5	1.7	0.3	1.2	0.9	3.3
Senecionine-*N*-oxide	SnNO	0.4	1.3	0.6	2.1	1.8	6.5
Seneciphylline	Sp	1.5	5.4	0.3	1.1	1.5	5.4
Seneciphylline-*N*-oxide	SpNO	0.9	3.3	0.4	1.4	2.2	8.0
Senecivernine	Sv	0.5	1.7	0.3	1.2	1.5	5.4
Senecivernine-*N*-oxide	SvNO	0.4	1.3	0.6	2.1	2.2	8.0
Senkirkine	Sk	0.4	1.5	0.3	0.9	1.0	3.6
Trichodesmine	Td	0.5	1.6	0.4	1.3	2.1	7.7

8.2.2. Recovery and repeatability

The recovery of the PAs from (herbal) tea infusion was determined by the analysis of eight replicates of blank mixed herbal and rooibos tea fortified at a level of 0.267 µg/L (corresponding to 20 µg/kg dry tea). The results are shown in Table 18. Additionally, recovery was determined by a two-fold analysis at a concentration level of 2 µg/L (corresponding to 150 µg/kg dry tea). The mean recovery rates were

between 77 % for senecionine and 111 % for intermedine with a repeatability of 8-20 % (data not shown).

Table 18: Recovery data of individual PAs for mixed herbal tea (n = 5) and rooibos tea (n = 3)

		Mixed herbal tea		Rooibos tea	
Pyrrolizidine alkaloid	**Abbr.**	**Recovery (%)**	**RSD (%)**	**Recovery (%)**	**RSD (%)**
Echimidine	Em	102	11	78	9
Echimidine-*N*-oxide	EmNO	104	9	87	7
Erucifoline	Er	92	6	73	5
Erucifoline-*N*-oxide	ErNO	92	8	87	9
Europine	Eu	106	5	72	7
Europine-*N*-oxide	EuNO	106	8	98	9
Heliotrine	He	105	5	80	6
Heliotrine-*N*-oxide	HeNO	103	6	115	11
Intermedine	Im	111	5	82	7
Intermedine-*N*-oxide	ImNO	96	7	98	9
Jacobine	Jb	110	6	84	10
Jacobine-*N*-oxide	JbNO	97	11	87	8
Lasiocarpine	Lc	93	14	76	6
Lasiocarpine-*N*-oxide	LcNO	93	38	79	11
Lycopsamine	Ly	103	10	81	7
Lycopsamine-*N*-oxide	LyNO	106	6	112	10
Monocrotaline	Mc	106	6	77	7
Monocrotaline-*N*-oxide	McNO	102	5	91	11
Retrorsine	Re	62	5	74	6
Retrorsine-*N*-oxide	ReNO	92	16	82	7
Senecionine	Sn	95	17	72	11
Senecionine-*N*-oxide	SnNO	91	8	83	6
Seneciphylline	Sp	92	9	72	11
Seneciphylline-*N*-oxide	SpNO	100	6	85	5
Senecivernine	Sv	80	7	72	8
Senecivernine-*N*-oxide	SvNO	96	6	84	8
Senkirkine	Sk	94	12	73	10
Trichodesmine	Td	100	20	72	2

As comparatively high concentrations were expected for food supplements derived from dried plant products and bee pollen, a spiking level at 80 µg/kg was chosen. Since lower concentrations were expected in oil-based supplements, the recovery samples were spiked at 6 µg/kg. Average recovery and repeatability for dry food supplements were determined by the analysis of seven replicates of blank samples spiked with PA mix, while for bee products and oil-based supplements two replicate analyses of blanks spiked with PA mix were performed. Results are shown in Table 19. The mean recovery rates for dry food supplements were between 85 % for erucifoline and 107 % for monocrotaline-*N*-oxide, with a repeatability of 3-17 %. For the bee products good recoveries (79-106 %) and repeatability (0-15 %) were obtained as well. For oil-based supplements, repeatability was good (1-11 %), but recovery was more variable, ranging from 28 % for seneciphylline-*N*-oxide to 104 % for europine-*N*-oxide. Besides seneciphylline-*N*-oxide, also senecionine-*N*-oxide (34 %) and senecivernine-*N*-oxide (35 %) gave low recoveries.

Table 19: Recovery data of individual PAs for the three different types of (herbal) food supplements

Pyrrolizidine alkaloid	Abbr.	Dry herbal supplements [(a)]		Bee product supplements [(b)]		Oil-based supplements [(b)]	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Echimidine	Em	93	7	98	2	72	8
Echimidine-*N*-oxide	EmNO	97	6	106	2	92	3
Erucifoline	Er	85	3	93	5	83	3
Erucifoline-*N*-oxide	ErNO	105	5	91	12	100	2
Europine	Eu	102	8	95	7	83	3
Europine-*N*-oxide	EuNO	104	5	94	9	104	6
Heliotrine	He	98	7	84	12	94	11
Heliotrine-*N*-oxide	HeNO	97	6	88	10	103	10
Intermedine	Im	102	14	83	13	84	2
Intermedine-*N*-oxide	ImNO	104	6	88	16	102	4
Jacobine	Jb	89	7	85	10	82	1
Jacobine-*N*-oxide	JbNO	102	11	91	12	103	2
Lasiocarpine	Lc	96	5	84	1	84	1
Lasiocarpine-*N*-oxide	LcNO	104	6	91	9	95	8
Lycopsamine	Ly	92	17	91	9	64	6
Lycopsamine-*N*-oxide	LyNO	107	8	91	12	90	4
Monocrotaline	Mc	91	9	91	4	85	1
Monocrotaline-*N*-oxide	McNO	107	8	92	4	98	4
Retrorsine	Re	98	8	79	15	80	8
Retrorsine-*N*-oxide	ReNO	103	6	86	10	78	2
Senecionine	Sn	85	16	81	1	69	3
Senecionine-*N*-oxide	SnNO	87	6	104	0	34 [(c)]	2
Seneciphylline	Sp	87	6	82	6	67	6
Seneciphylline-*N*-oxide	SpNO	99	4	99	1	28 [(c)]	10
Senecivernine	Sv	86	17	80	2	68	3
Senecivernine-*N*-oxide	SvNO	101	6	104	0	35 [(c)]	2
Senkirkine	Sk	96	6	91	1	74	3
Trichodesmine	Td	95	4	81	14	84	5

(a): N = 7.
(b): N = 2.
(c): Recovery outside the preferred range of 60-120 %.

8.2.3. Linearity

The verification of linearity within the concentration range used for the analysis of PAs in (herbal) tea infusion (0.013-4.000 µg/L; corresponding to 1-300 µg/kg dry tea) was based on the goodness-of-fit-test according to Mandel (DIN, 1986). A detailed description of the calculations can be found in Appendix B.

The calibration data obtained in a representative herbal tea infusion are shown in Appendix B. The coefficient of determination (R^2) for the nine-point calibration curves ranged from 0.995 (for monocrotaline-*N*-oxide) to 0.990 (for senecionine-*N*-oxide). For each analyte (except retrorsine-*N*-oxide) a test value below the required reference value was obtained. For retrorsine-*N*-oxide, as the R^2 of the calibration curve was still above 0.99, a linear regression was assumed. Therefore, the calibration curves for all analytes were considered to be linear over a concentration range of 0.013-4.000 µg/L.

Some representative calibration curves in concentrated tea extract are shown in Figure 5: Calibration curves for PA FBs comprising monoesters (lycopsamine, heliotrine), open chain diesters (echmidine, lasiocarpine) as well as cyclic diesters (senecionine, retrorsine, senkirkine) and PANOs (retrorsine-*N*-oxide, lasiocarpine-*N*-oxide) were linear over the full calibration range.

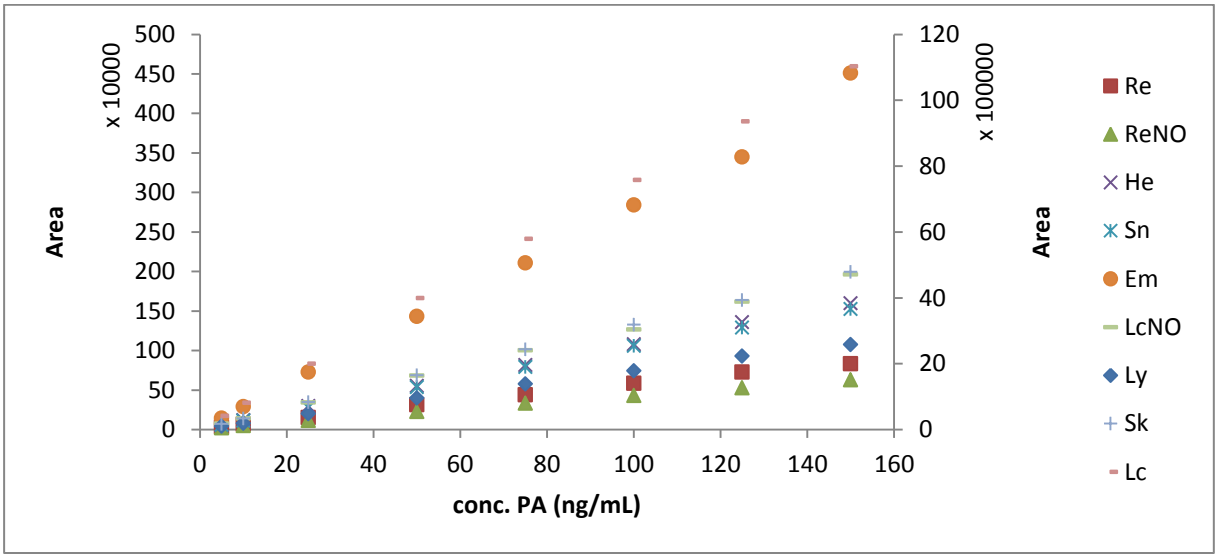

Figure 5: Calibration curves for selected PA matrix-matched standards within the concentration range of 1-150 ng/mL in concentrated herbal tea extract, representing a PA concentration range of 0.027-4.000 µg/L in tea infusion. Abbreviations are explained in Table 11.

Figure 6 shows the LC-MS/MS chromatograms of a blank tea sample (a mixture of peppermint, chamomile, caraway and fennel) spiked with a mixture of PA standards. In general, the number of interfering matrix peaks is limited allowing the determination of PAs with high specificity and high sensitivity.

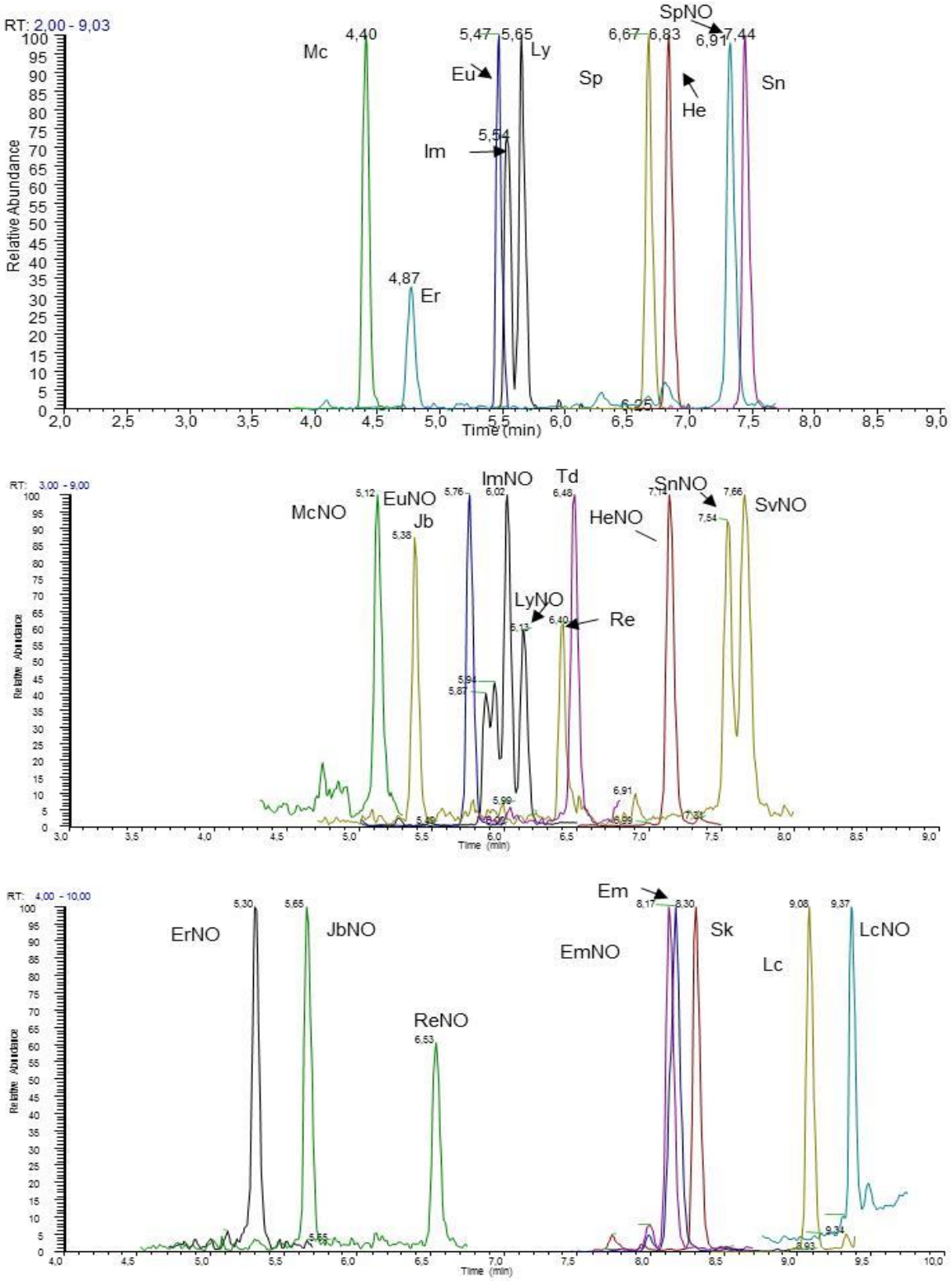

Figure 6: LC-MS/MS chromatograms of a blank mixed-herbal tea sample spiked with a mixture of PAs (20 µg/kg). For each PA the quantifier ion is shown. Abbreviations are explained in Table 11.

8.2.4. Validation by collaborative trial

The method for the determination of PAs in herbal tea has also been validated by a collaborative validation study which in 2013 has been organised by BfR according to the standard ISO/IUPAC/AOAC (Horwitz, 1995; AOAC, 2002). The results of the collaborative study revealed a good reproducibility of the method, good recoveries (trueness of the method) and the criteria for a successful validation have been met. The final study report is available from the BfR website (BfR, 2015).

9. COMMUTABILITY OF THE LC-MS/MS METHODS: RIKILT AND BFR

The commutability of the two LC-MS/MS methods used within the framework of this project was evaluated (for responsibilities refer to Table 1). A standard mixture containing the PAs (100 ng/mL), prepared independently by RIKILT and by BfR was exchanged. The mixtures were compared by both institutes using their in-house validated methods. The comparison of the standards provided the same outcome with the two LC-MS/MS methods used. The relative concentration in the mixture varied from 44 % (seneciphylline) to 175 % (senecionine). The RIKILT standard mix gave for a number of compounds (monocrotaline, europine, seneciphylline, senecionine, lasiocarpine, seneciphylline-*N*-oxide, lasiocarpine-*N*-oxide) lower concentrations (more than 20 % lower) than the one prepared by BfR. The BfR standard mix gave for Sn a significantly lower concentration. Some discrepancies in the results could be explained by co-elution of PAs in the methods used by either RIKILT or BfR (integerrimine/senecivernine and integerrimine-*N*-oxide/senecionine-*N*-oxide in the case of the BfR method, and lycopsamine/intermedine and lycopsamine-*N*-oxide/intermedine-*N*-oxide in the case of the RIKILT method).

Further research was conducted to find out the origin of the bias and was designed to evaluate (i) the commutability of the current RIKILT mixed standard solution with the one prepared in 2013; (ii) the influence of purity of the standards (i.e. the possible addition of responses due to impurities or mixtures; (iii) the influence of the amount of standard weighed during the preparation of the individual stock solutions; and (iv) the influence of differences between lots and between suppliers. The following results were obtained:

(i) The standard mixture prepared and used by RIKILT in 2013 for PAs analyses was compared with the standard prepared for the current project. No significant deviations (exceeding 20 %) were found (data not shown).

(ii) The purity of each individual PA standard was verified by BfR in order to evaluate the possible addition of responses due to impurities or mixtures. This experiment consisted of injecting the individual standard solutions used to prepare the standard mix and verifying that only the peak of the analye of interest was detected and quantified. The results revealed no significant contamination of individual PAs to the response of other PAs, with the exception of europine that contained around 8 % of heliotrine (Appendix B).

(iii) The influence of the amount of standard weighted during the preparation of the individual stock solution was also evaluated by BfR. Due to low amounts available and its cost, between 1-2 mg had been weighed to prepare the standards, which might entail a relatively high uncertainty due to the small amount weighed. In this experiment, when possible, a higher amount of individual standards were weighed to prepared individual stock solutions. The final standard mixture was prepared with the new standards and compared with the RIKILT standard. The results (see Appendix C) revealed that, although the differences in response were reduced for some compounds, such as lycopsamine-*N*-oxide or trichodesmine, the outcomes were not significantly different for most of the individual standards.

(iv) Finally, differences between lots and between suppliers were investigated. This test was conducted with senecionine, the PA with the highest deviation in the test and an important compound because it was often present in the (herbal) teas that were collected (Bodi et. al 2013). The experiment consisted of comparing the responses of senecionine for two different lots of the same supplier (Roth) and for one different supplier (Phytolab). The results revealed a difference of around 10 % for the same supplier and different lot, but around 20 % difference between suppliers, which might explain some of the initial differences found between RIKILT and BfR.

10. INTER-LABORATORY PERFORMANCE COMPARISON: RIKILT AND IRTA

An inter-laboratory exercise to assess the performance of IRTA in the sample preparation of the extracts of animal-derived products was carried out. The samples for quality purposes, which included the samples of the inter-laboratory exercise, were shipped to IRTA. IRTA conducted the extractions for the different matrices (milk, eggs, poultry meat and beef meat) and sent the extracts back to RIKILT for LC-MS/MS analyses. At the same time, RIKILT conducted the extraction of a similar set of samples of milk, eggs and poultry meat matrices. Due to time limitations, RIKILT did not conduct the extraction of beef meat samples. The results for milk and eggs were satisfactory with respect to recovery and accuracy (Table 20) (full data are shown in Appendix C).

Table 20: Summary of the performance of IRTA and RIKILT in the inter-laboratory exercise: recovery and accuracy (average of 35 individual PAs)

Matrix	Laboratory	Average recovery (%)	RSD recovery (%)	Accuracy (%)	RSD accuracy (%)
Milk (2.5 µg/kg)	IRTA	96.0	2.5	98.6	12.3
	RIKILT	91.6	4.0	101.0	4.0
Eggs (10 µg/kg)	IRTA	85.5	2.4	99.7	2.9
	RIKILT	76.7	3.5	91.9	10.7
Poultry meat (5 µg/kg)	IRTA	49.1	6.0	99.6	1.9
	RIKILT	63.5	3.7	112.9	3.7
Beef meat (5 µg/kg)	IRTA	52.3	2.4	102.6	4.0

Although good accuracy was obtained for the test extraction of poultry and beef meat samples, the average recovery for both matrices was only around 50-60 %, which is lower than the average of 80 % obtained during the in-house validation at RIKILT (see Table 15). The origin of this bias could not be fully clarified. Degradation of PAs in the QC samples during storage could be one of the contributing factors (this will be discussed in more detail in Section 11.1). Increased matrix suppression could be another contributing factor to the lower average recovery. Occasionally, clogging of SPE cartridges due to excessive particulates in the sample extract was observed as well. For these reasons it was decided to reduce the amount of meat sample extract used for SPE clean-up from 10 mL (as was used during the in-house validation) to 5 mL. The amended protocol (see Section 5.1.3) was used for all meat samples analysed during this survey.

11. ON-GOING QUALITY CONTROL DURING THE ANALYSIS OF SAMPLES

11.1. Quality control during the analyses of PAs in animal-derived food products

11.1.1. Quality control data obtained during the study

The method performance with regard to routine recovery was monitored during the measurement period by the analysis of the QC samples included in each series. The results are shown in Figure 7 (milk), Figure 8 (eggs) and Figure 9 (meat) for the 35 PAs under study.

The milk samples were analysed in 10 separate runs (between June 17, 2014 and May 2, 2015). The recovery values for the QC samples spiked at 2.5 µg/L indicate that for almost all PAs acceptable recoveries between 60 and 130 % were obtained (Figure 7). No obvious trends could be detected, indicating a sufficient stability of the QC samples prepared before the start of the analyses and during storage at -20 °C until analysis. The mean recovery obtained for the compounds was 86.7 % (SD: 12.4 %). The lowest mean recovery was observed for riddelliine-*N*-oxide (64.4 ± 16.7 %) and the highest mean recovery was obtained for florosenine (102.1 ± 25.2 %). Overall, the results obtained were in accordance with the validation parameters obtained previously (see Section 8.1.2).

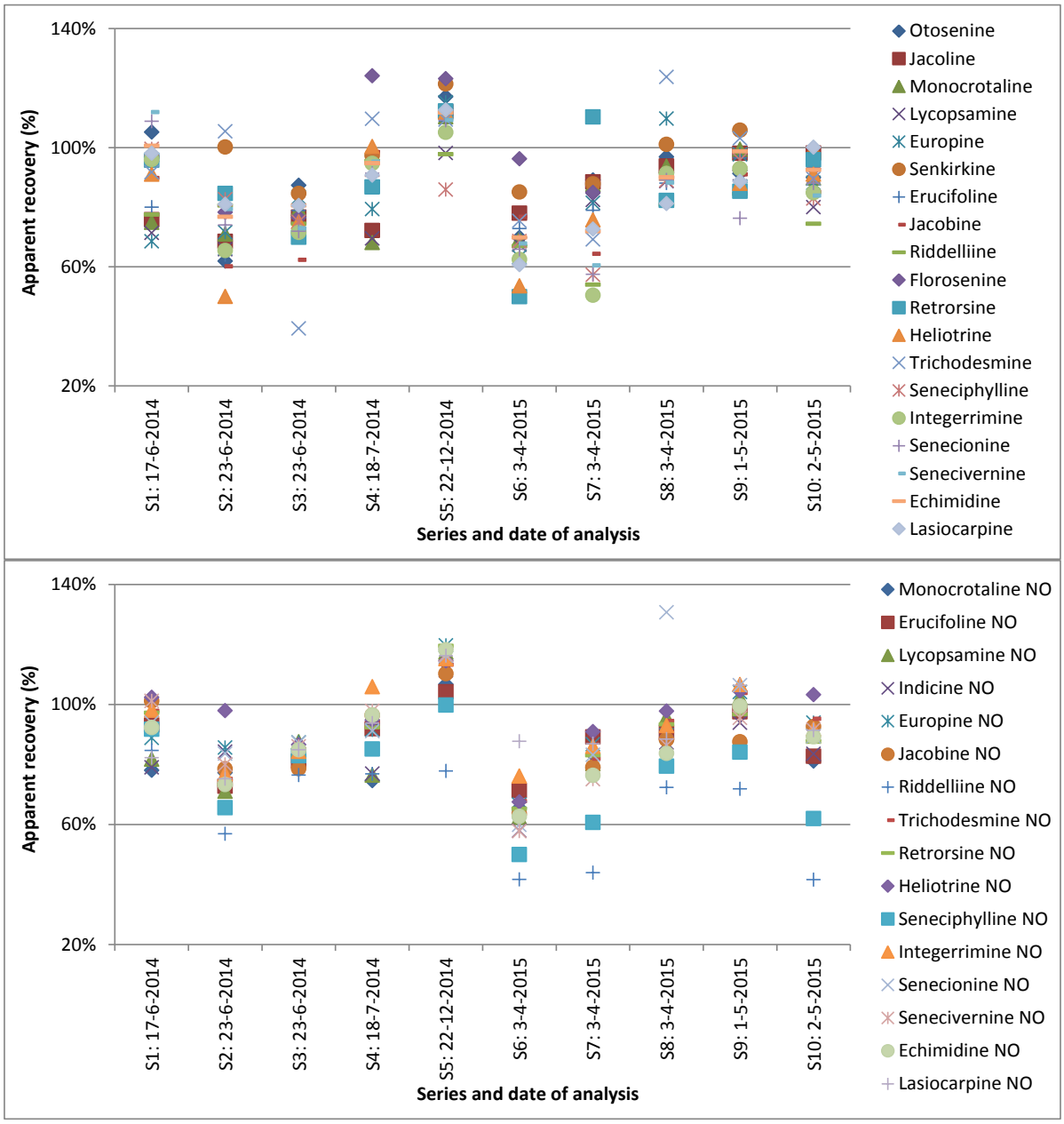

Figure 7: Analysis of milk samples: Recovery of the PA free bases (above) and *N*-oxides (below) in the QC samples (2.5 µg/L) prepared at the start of the project, obtained during the first sampling period (series S1-S4) and second sampling period (series S5-S10)

The egg samples were analysed in 9 separate runs (between July 7, 2014 and May 2, 2015). The recovery values for the QC samples spiked at 10 µg/kg indicate that for the large majority of PAs acceptable recoveries between 60 and 90 % were obtained (Figure 8). No obvious trends could be detected, indicating a sufficient stability of the QC samples prepared before the start of the analyses and during storage at -20 °C until analysis. The mean recovery obtained for the compounds was 74.6 % (SD: 5.3 %). The lowest mean recovery was observed for riddelliine-*N*-oxide (55.3 ± 14.2 %) and the highest mean recovery was obtained for senecionine-*N*-oxide (85.9 ± 6.5 %). Overall, the results obtained were in accordance with the validation parameters obtained previously (see Section 8.1.2).

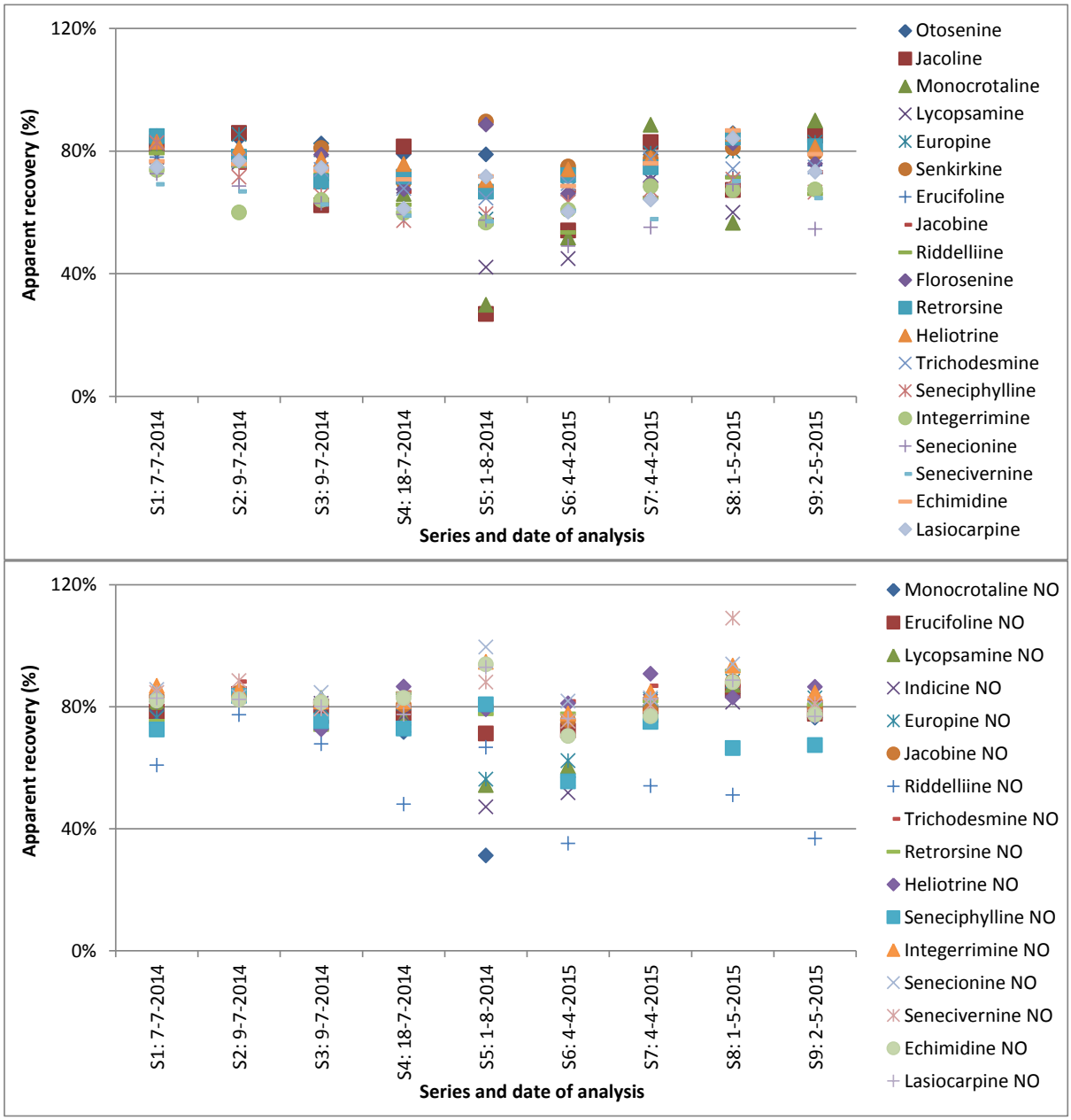

Figure 8: Analysis of egg samples: Recovery of the PA free bases (above) and *N*-oxides (below) in the QC samples (10 µg/kg), prepared at the start of the project, obtained during the first sampling period (series S1-S5) and second sampling period (series S6-S9)

The meat samples were analysed in 15 separate runs (between July 18, 2014 and April 15, 2015). The recovery values for the QC samples spiked at 5 µg/kg of the first 9 series (run during the first sampling period, in July and August, 2014), indicated that in general modest to even low recoveries (down to only 10 %) in case of some PANOs were obtained (Figure 9). These recoveries were much lower than that had been obtained during the in-house validation of the method (see Section 8.1.2). It also appeared as if for most PAs a decline of the recovery over time occurred. On average the recovery of the third analytical series of a particular meat matrix was 10 % lower than in the first series (for beef meat the difference was even 20 %). The results indicated that the stability of the QC samples, prepared before the start of the analyses (see Section 4.2.1) and stored at -20 °C until analysis, was rather limited. The consequence of this decline is that in case of positive findings, a potential overestimation of the PA content in the samples analysed could occur.

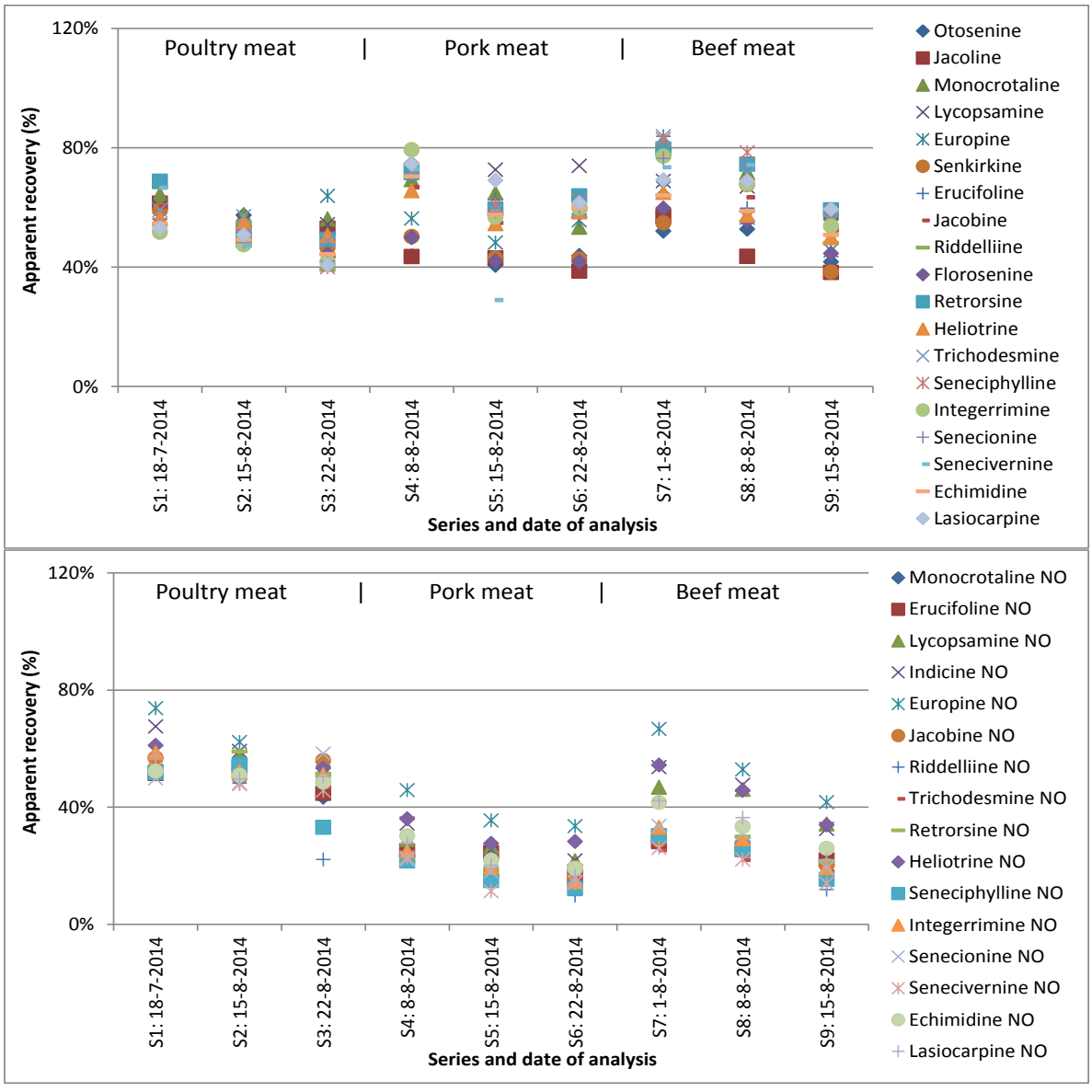

Figure 9: Analysis of meat samples: Recovery of the PA free bases (above) and *N*-oxides (below) in the QC samples (5 µg/kg), prepared before the start of the project, obtained during the first sampling period. Series S1-S3 are poultry meat, series S4-S6 are pork meat and series S7-S9 are beef meat.

Based on these results it was decided that the QC (and MMS) samples for meat should not be used for quality control of the analytical series during the second sampling period. Instead it was decided that blank meat samples were to be spiked on the spot for quality control and matrix-matched calibration. The recovery results obtained with fresh QC samples for the 6 series of meat analysed during the second sampling period are shown in Figure 10. The recovery data for the QC samples indicate that for the large majority of PAs acceptable recoveries between 70 and 120 % were obtained. However, one of the two series of bovine meat (series 5 in Figure 10), clearly showed a larger variability in the results than in the other series run with freshly prepared MMS and QC samples. The results indicate that bovine meat is a particularly difficult matrix with respect to the analysis of PAs, behaving differently from the poultry and pork matrices.

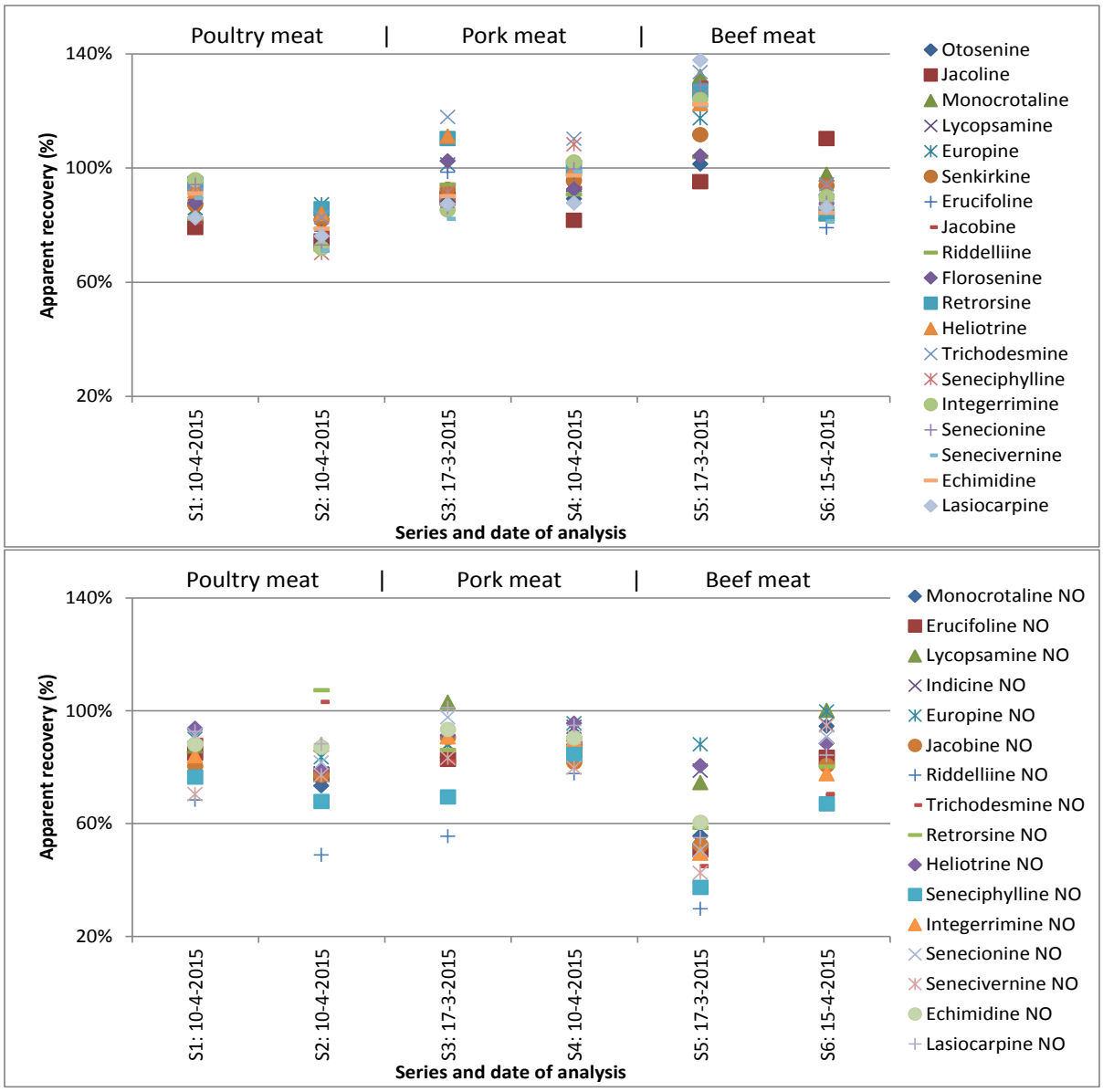

Figure 10: Analysis of meat samples: Recovery of the PA free bases (above) and *N*-oxides (below) in the QC samples (5 µg/kg), prepared during the analytical series of the second sampling period. Series S1-S2 are poultry meat, series S3-S4 are pork meat and series S5-S6 are beef meat.

11.1.2. Mid-term and long-term stability of QC samples

For the mid-term (7 months) and long-term (15 months) stability tests for storage at -20 °C and at -80 °C, stability samples (containing 5 subsamples each) for each matrix (milk, egg, bovine meat, porcine meat, poultry meat) were prepared at the beginning of the project (February 2014). After 7 months of storage, one set of stability samples for each matrix stored at -20 °C and at -80 °C was taken from the freezer and analysed for their PAs content. In May 2015, at the end of the project and after 15 months of storage, a second set of stability samples for each matrix stored at -20 °C and at -80 °C was taken from the freezer and analysed for their PA content. The results are summarised in Table 21.

Table 21: Mid-term (7 months) and long-term (15 months) stability results for samples stored at -20 °C compared to samples stored at -80 °C. Average loss or gain (%) of the free bases (PA FBs) and *N*-oxides (PANOs) in spiked samples. RSD = average relative standard deviation in the measurements of samples stored at -20 °C and at -80 °C.

Matrix	Spike level (µg/kg)	PA FBs			PANOs		
		Recovery (-20 °C, %)	RSD (-20 °C)	RSD (-80 °C)	Recovery (-20 °C, %)	RSD (-20 °C)	RSD (-80 °C)
Mid-term (7 months)							
Milk	2.5	97.3 %	9.6 %	3.4 %	111.4 %	7.1 %	3.7 %
Eggs	10	92.6 %	3.5 %	2.5 %	94.7 %	2.3 %	6.8 %
Beef meat	5	80.3 %	7.0 %	8.5 %	59.7 %	31 %	35 %
Pork meat	5	86.8 %	6.3 %	10.8 %	88.6 %	12.2 %	34 %
Poultry meat	5	86.6 %	9.8 %	3.6 %	84.8 %	8.5 %	4.1 %
Long-term (15 months)							
Milk	2.5	110.8 %	3.5 %	8.0 %	100.5 %	2.8 %	10.4 %
Eggs	10	107.8 %	3.9 %	5.5 %	96.7 %	2.8 %	4.4 %
Beef meat	5	74.3 %	3.5 %	3.1 %	63.4 %	24 %	8.8 %
Pork meat	5	90.6 %	8.2 %	4.7 %	71.4 %	29 %	10.8 %
Poultry meat	5	79.8 %	13.5 %	8.4 %	68.4 %	13.8 %	8.4 %

The results of the stability samples showed that PA FBs and PANOs were stable in eggs and milk for the whole period of the study (recovery > 90 % after 15 months). However, for the different meat matrices, after 7 and 15 months of storage at -20 °C lower average recoveries (< 90 %) for PA FBs and PANOs were obtained than for meat samples stored at -80 °C. For PANOs, after 15 months of storage at -20 °C, recoveries in meat were in the order of 60-70 %, while slightly higher recoveries were obtained for PA FBs (75-90 %). The recovery loss was most significant for the PANOs in bovine meat (average recovery loss of 40 % after 7 and 15 months). Furthermore there was often (but not always) a substantial variation in the results for the PANOs in bovine and porcine meat, both for the samples stored at -20 °C as the samples stored for 7 months at -80 °C. Consequently, due to the apparent limited stability of the QC meat samples stored at -20 °C, in combination with the results obtained for the QC samples during the ongoing quality control (Section 11.1.1), it was decided not to use the MM(R)S and QC samples for the second sampling round. For the analysis of meat samples collected during the second sampling round, blank meat samples were to be spiked on the spot for MMS and quality control.

The fact that there was also a high variation in the results of the porcine and bovine mid-term stability samples stored at -80 °C, may be an indication that the variability is linked to the freezing/thawing process of the meat. The exact fate of PANOs in bovine meat remains unclear, and it could be related to specific constituents of the beef meat matrix (e.g. metal ions that can cause degradation of PANOs).

11.1.3. Short-term stability of PAs in meat stored under retail conditions

As described in Sections 11.1.1 and 11.1.2, it was observed that for the meat matrices upon storage at -20 °C a decay in the recovery percentages of the spiked analytes occurred over time, especially affecting PANOs. The recoveries for these PANOs were much lower than the ones obtained during the in-house validation of the method. The results indicated that the stability of the MMS and QC samples, although being stored at -20 °C, was limited. The results also raised questions regarding the stability of PAs in meat samples as such, because in retail shops the food products are typically stored under cooled conditions (4-6 °C) for several days. It cannot be excluded that enzymatic and microbiological conversions including degradation of PAs will take place in meat products under these 'normal' storage conditions. A breakdown of PAs during storage in the supermarket before the product is acquired, could also explain the absence of PAs in any of the meat products analysed during the first sampling period. No studies are known in which the effect of storage and storage temperature on the stability of PAs in animal matrices has been investigated. However, enzymatic degradation of residues in meat and liver tissues during storage is a well-known phenomenon for several classes of antibiotics, such as penicillins and tetracyclines (van Holthoon et al., 2010; Berendsen et al., 2011).

Taken into account these findings, a more detailed investigation on the stability of PAs in meat samples was undertaken. An assessment of the short term stability of PAs in meat samples (poultry, pork and beef) under storage conditions used in supermarkets and retail shops (at 4-6 °C for up to eight days) was made. For the short term stability test at 4-6 °C, freshly bought blank poultry, pork and beef meat materials were homogenised and subsamples (3 g) of the three materials were individually spiked in triplicate at 1 µg/kg and at 10 µg/kg and stored in the refrigerator (4-6 °C). Samples were taken at different time points (0, 1, 2, 4, and 8 days) and stored at -20 °C until analysis of their PA content, which was conducted 3 days after the end of the experiment. Sample preparation and analysis was performed according to the protocol for meat (Section 5.1.3).

The results of the short-term stability are shown in Appendix E. Table E.1 and Table E.2 show a summary of the results for short-term stability of PAs free bases and PANOs in meat stored at 4-6 °C. The results revealed that there was no significant degradation of the PA FBs and PANOs, both at high (10 µg/kg) and low (1 µg/kg) level of spiking during the time span of the experiment. Also at the level of individual compounds no significant trends were found (data not shown). This indicates that in any case there is no substantial metabolic conversion of PAs at the meat surface. However, at the same time it was noticed that, in particular for the PANOs spiked to the beef meat samples, a much higher variation in the results was obtained, both at the high (10 µg/kg) and low (1 µg/kg) level of spiking. In contrast, the variation in results was low for the poultry meat samples and for the porcine meat it was intermediate.

The high variation in results for PANOs in beef meat in part can be explained by a much stronger suppression of mass spectrometric signals for many of the PANOs (Table E.3). From Table E.3 it follows that the average suppression for the PA FBs ranged from 15 % in bovine meat to 30 % in poultry meat. The matrix suppression for the PANOs in pork and poultry meat was on average 20 %. However, for beef meat an average suppression of 80 % was observed for the PANOs. Apparently, bovine meat contains matrix components that strongly interfere with the measurement of the PANOs. The nature of these matrix components is unknown and the results show that these components are not adequately removed by the sample clean-up procedure.

11.2. Quality control during the analyses of PAs in plant-derived food products

The method performance for (herbal) tea analysis with regard to routine recovery has been monitored and is shown in Figure 11 for the main PAs during the period of sample analyses. All herbal teas

(including mixed herbal teas, chamomile, peppermint and fennel) were analysed in sequence 1-8. For the quantification matrix-matched standards and a recovery sample were prepared from blank material of mixed herbs according to Section 4.2.2. The rooibos teas were analysed in sequence 9. For the quantification matrix-matched standards and a recovery sample were prepared by using a blank rooibos tea (also according to Section 4.2.2). This procedure includes a correction of the results for matrix effects that might occur during MS detection. No correction of recovery of the sample preparation was applied. The analysis of green and black tea was performed with slight differences regarding the SPE procedure (as described in Section 5.2.1) and the quantification approach. Both modifications were established because of lower recovery rates compared to herbal teas and strong matrix effects in the fermented materials like black and green teas. For quantification a five point matrix calibration (by means of spiking of blank material prior to sample preparation/extraction) was prepared. This procedure includes a correction for recovery of sample preparation as well as matrix effects.

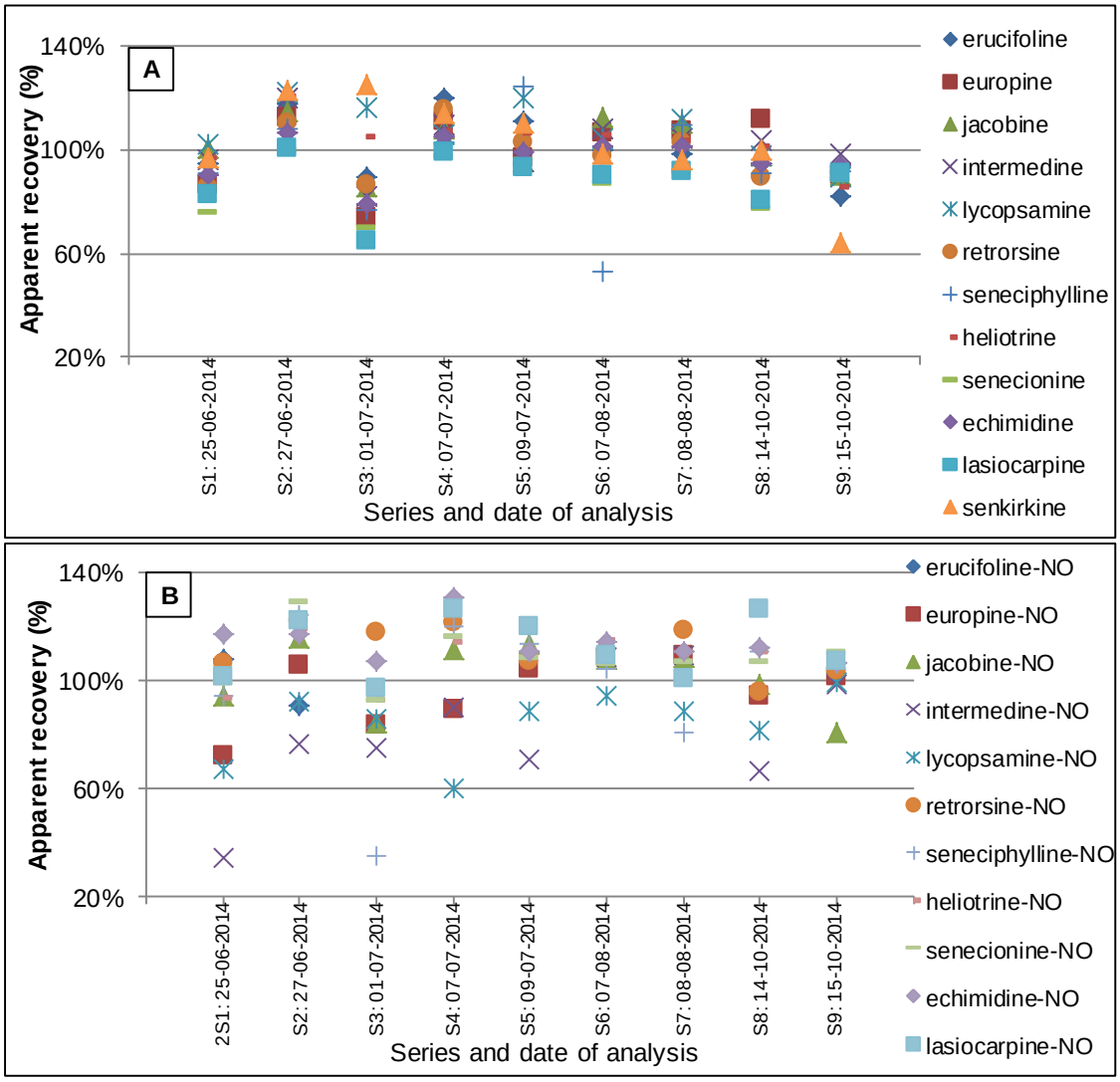

Figure 11: Quality control charts, showing (A) the recoveries of the PA free bases and (B) their *N*-oxides in the spiked blank tea infusion samples (0.267 µg/L, corresponding to 20 µg/kg in dry tea). Each spot represents the individual recovery per analysis (series S1-S8 herbal teas, series S9 rooibos teas.

The food supplements samples were analysed in 10 separate runs (between February 11 and March 14, 2015). The recovery data (Figure 12) indicate sufficient recoveries (55-120 %). Mean recoveries ranged between 77 and 97 % and therefore demonstrate a sufficient method performance for the analysis of investigated teas. In few cases (seneciphylline, seneciphylline-*N*-oxide and intermedine-*N*-oxide) a relatively low recovery (25-55 %) was obtained, but this had no major impact on the quality of the obtained results.

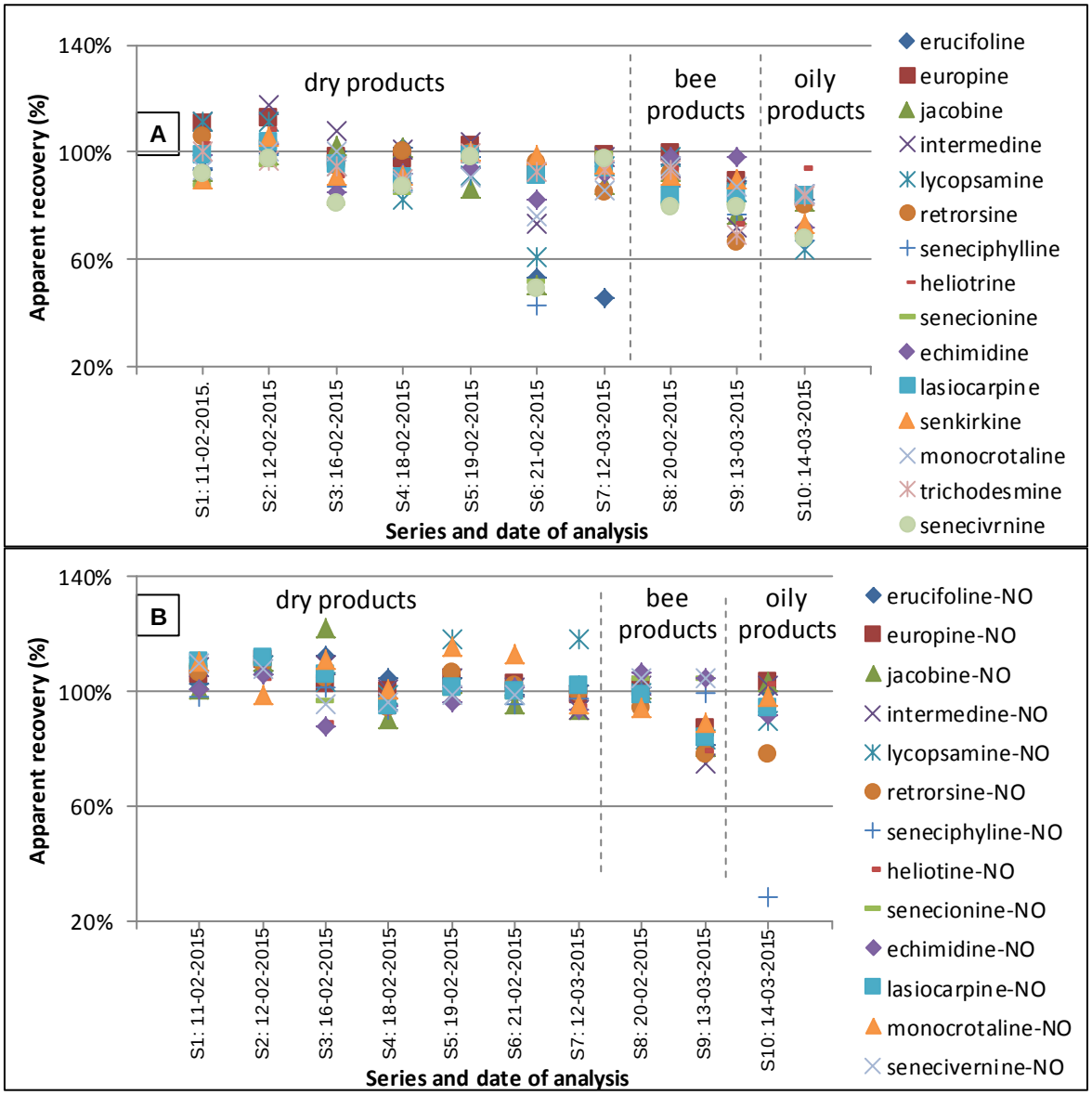

Figure 12: Quality control charts showing (A) the recoveries of the PAs and (B) their *N*-oxides in the spiked blank samples. Dry food supplements and food supplements containing bee products were spiked at 80 µg/kg. Oil-based supplements were spiked at 6 µg/kg. Each spot represents the individual recovery per analysis (series S1-S7: dry food supplements, series S8-S9: bee products, series S10: oil-based food supplements).

12. UNCERTAINTY ANALYSIS OF THE APPLIED METHODOLOGY

The following factors can been identified that may contribute to the measurement uncertainty of the reported results: (i) impurities present in the analytical standards used and differences between the actual purity of the standards compared to the purity reported by the supplier(s) (see Section 4.1), (ii) differences in the PA standard mix concentrations prepared by RIKILT and BfR due to weighting or other errors made (see Section 9), (iii) instability of PAs during storage under retail conditions, or during storage at -20 °C until sample preparation (relevant for animal-derived samples, see Section 11.1), (iv) sample- and PA-specific matrix effects resulting in suppression and/or enhancement of the mass spectrometric signal (Sections 8 and 11), (v) variability in extraction efficiency and recovery (Sections 8 and 11), (vi) variability in instrument sensitivity and signal linearity during measurement. Based on the QC data obtained (Section 11), and depending on the specific combination of PA, matrix and concentration, a measurement uncertainty in the range of 30-50 % may reasonably be estimated.

13. SAMPLES COLLECTED

13.1. Animal-derived products

A summary of the final sampling plan and the final number of collected items per food product is shown in Table 22. All samples were purchased from food retails. In total 746 samples have been purchased, which is 7 % more than the number proposed at the start of the project (see Table 2, Section 1). For all three major food categories a slightly higher number of samples were collected than proposed. A total of 120 samples of organic production were collected, corresponding to 16 % of the total. At the start of the project only 75 samples of organic production (11 %) were foreseen. The percentage of organic samples was highest in the category of milk and milk products, but also organic eggs and meat samples were readily available in retail shops.

Table 22: Overview of animal-derived food samples collected and analysed

	Target number of samples	Samples collected and analysed	Organic samples	% Organic
All animal-derived food products	**700**	**746**	**120**	**16 %**
Milk and milk products	**250**	**268**	**52**	**19 %**
Pasteurised and UHT milk (skimmed, semi-skimmed, whole milk)	175	182	44	24 %
Cow milk, 3-4 % fat		*55*	*12*	*22 %*
Cow milk, 1-2.9 % fat		*76*	*23*	*30 %*
Cow milk, <1 % fat		*38*	*4*	*11 %*
Goat milk		*13*	*5*	*38 %*
Fermented milk products	25	27	3	11 %
Yoghurt, cow milk, >3 % fat		*16*	*2*	*13 %*
Yoghurt, cow milk, <1 % fat		*11*	*1*	*9 %*
Cheese	25	34	2	6 %
Gouda		*19 (a)*	*1*	*5 %*
Brie		*15 (b)*	*1*	*7 %*
Milk powder (infant formula)	25	25	3	12 %
Infant formula, milk-based, powder (0-6 months)		*8*	*2*	*25 %*
Follow-on formula, milk-based, powder (6-36 months)		*17*	*1*	*6 %*
Eggs and eggs products	**200**	**205**	**30**	**15 %**
Fresh eggs	200	205	30	15 %

	Target number of samples	Samples collected and analysed	Organic samples	% Organic
Meat and meat products	**250**	**273**	**38**	**14 %**
Beef meat	75	80	14	18 %
Pork meat (filet)	75	79	10	13 %
Poultry meat (chicken breast filet)	70	83 (c)	12	14 %
Liver	30	31	2	6 %
Beef liver	*10*	*11*	*1*	*9 %*
Pork liver	*10*	*10*	*0*	*0 %*
Chicken liver	*10*	*10 (d)*	*1*	*10 %*

(a): Including 4 samples of Emmental cheese.
(b): Including 1 sample of Camembert cheese.
(c): Including 5 samples of turkey meat.
(d): Including 1 sample of turkey liver.

Figure 13 shows an overview of the number of animal-derived products collected over the course of the survey. Samples were collected from January 2014 till April 2015 in two major sampling rounds. During the first sampling period (January to July 2014) a total of 396 products were collected and during the second sampling period (October 2014 to April 2015) another 350 products. The majority of products had a short shelf-life (1-2 weeks), thus the time of sampling reflects the time of production, covering the different seasons, except part of the summer period.

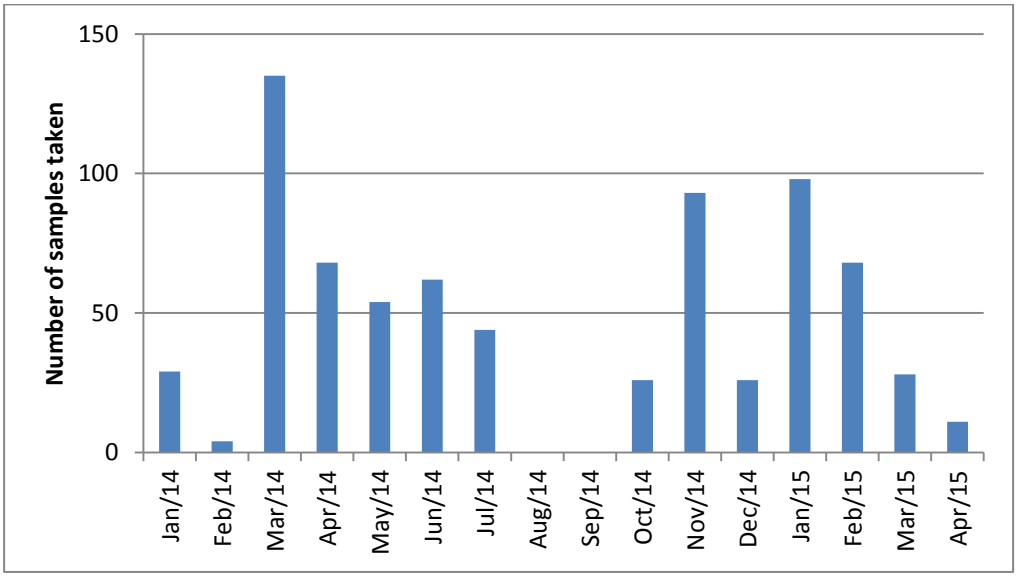

Figure 13: Overview of the monthly number of products collected of animal origin

An overview of the country of production of the animal-derived food samples collected is shown in Table 23. Over 60 % of the food samples were produced in Germany, the Netherlands and Spain, which were also the main sampling countries in this survey. It can be concluded that the majority of the food products sold in the retail shops is from national production. A smaller part may come from a neighbouring country (e.g. Belgium or German products sold in the Netherlands). Products from outside the European Union were not found in the retail outlets that were sampled.

Table 23: Overview of the country of production of the animal-derived food samples collected

Country of production	Milk and milk products	Eggs	Meat and meat products	Total	% of total
Total	268	205	273	746	
Austria	1	-	3	4	0.5 %
Belgium	9	-	4	13	1.7 %
Czech Republic	1	-	-	1	0.1 %
Danmark	-	1	-	1	0.1 %
EU	1	-	-	1	0.1 %
France	43	26	41	110	14.7 %
Germany	69	39	60	168	22.5 %
Greece	16	20	15	51	6.8 %
Ireland	-	-	8	8	1.1 %
Italy	25	25	18	68	9.1 %
Netherlands	55	53	47	155	20.8 %
Poland	-	-	1	1	0.1 %
Portugal	3	-	-	3	0.4 %
Slovenia	-	-	1	1	0.1 %
Spain	37	41	71	149	20.0 %
Switzerland	2	-	-	2	0.3 %
Unknown	6	-	4	10	1.3 %

13.1.1. Milk and milk products

In this survey 268 samples of milk and milk products (including yoghurt, cheese and infant formula) were collected and analysed.

Milk. A total of 182 samples of milk were collected. The majority of samples were cow milk and a smaller proportion was goat milk (Table 24). Milk can be sub grouped in whole (3-4 % fat), semi-skimmed (1-3 % fat) and skimmed (<1 % fat). Goat milk in general is sold as whole milk. The consumption of goat milk is quite rare in the Southern Europe; hence the sampling was mostly conducted in the Netherlands and Germany. Buffalo milk, although quite popular in Italy, was not sampled because it is commonly used for the production of mozzarella and not for direct consumption. The sampling plan took into account the consumption habits[3] of each country regarding preferences on pasteurised ('fresh') milk or UHT ('sterilised') milk. Pasteurised milk represents the majority of the milk consumed in the Netherlands (80 %), while UHT milk is commonly preferred in France (97 %) and in Spain (95 %). Consumers in Italy, Greece and Germany slightly prefer UHT milk over pasteurised. Overall, 45 % of the milk samples collected was pasteurised and 55 % was UHT processed. Organic milk was available in all countries sampled, and 24 % of the samples collected were from organic production.

In the original sampling plan (Table 2) it was anticipated to collect 125 samples of cow and goat milk, of which 75 samples were to be taken during the first sampling period and 50 during the second period. Based on the results of the first sampling period, which yielded several positive samples, the number of samples collected during the second period was increased to 100, in order to obtain a larger set of samples analysed for the occurrence of PAs in milk.

[3] Elliot, Valerie. The UHT route to long-life planet. Sunday Times, 15 Oct 2007.

Table 24: Milk samples collected per country and type. The number of samples from organic production is given between brackets.

Country	Whole		Semi-skimmed		Skimmed		Goat		Total
	Past. [(a)]	UHT	Past.	UHT	Past.	UHT	Past	UHT	
Total	28 (8)	27 (4)	39 (14)	37 (9)	9 (2)	29 (2)	5 (2)	8 (3)	182 (44)
France	1 (-)	6 (1)	3 (1)	7 (1)	-	5 (1)	-	1 (-)	23 (4)
Germany	6 (2)	4 (1)	7 (3)	7 (3)	1 (-)	7 (-)	1 (-)	3 (3)	36 (12)
Greece	4 (2)	2 (-)	4 (2)	3 (-)	1 (-)	1 (-)	-	-	15 (4)
Italy	5 (2)	4 (-)	5 (2)	4 (-)	-	4 (-)	-	1 (-)	23 (4)
Netherlands	9 (1)	3 (-)	17 (6)	4 (2)	7 (2)	4 (-)	4 (2)	2 (-)	50 (13)
Spain	3 (1)	8 (2)	3 (-)	12 (3)	-	8 (1)	-	1 (-)	35 (7)

(a): Past. = pasteurised. UHT = Ultra-high temperature processed.

Yoghurt. The EFSA Food Consumption Database has three entries for yoghurts made from cow milk depending on their fat content. Due to relatively small number of samples to be collected it was decided to sample only yogurts with low (<1 %) fat content and yogurts with high (>3 %) fat content (Table 25). Furthermore, only plain yogurts - yoghurts without fruits or cereals - were collected.

Table 25: Yoghurt samples collected per country and type. The number of samples of organic production is given between brackets.

Sampling country	<1 % fat	>1 % fat	Total
Total	11 (1)	16 (2)	27 (3)
France	1 (-)	2 (-)	3 (-)
Germany	3 (-)	2 (1)	5 (1)
Greece	1 (-)	2 (1)	3 (-)
Italy	1 (-)	2 (-)	3 (-)
Netherlands	3 (1)	5 (1)	8 (2)
Spain	2 (-)	3 (-)	5 (-)

Cheese. Considering the number of samples planned to be collected (n = 25), and in order to make the sampling consistent from a statistical point of view, the sampling of cheese was limited to a number of popular soft and hard types, depending on the consumption habits. From the proposed soft cheese varieties (Camembert, feta, mozzarella) and hard cheese (Gouda and Emmental), it was decided to focus on one type of soft cheese and one type of hard cheese. As there are not many cheeses on the market that have wide range of production countries (most cheeses are only produced in one specific country), Brie was chosen as a representative of soft cheese, and Gouda as a representative of hard cheese. The number and type of cheese collected are given in Table 26.

Table 26: Cheese samples collected per country and type. The number of samples of organic production is given between brackets.

Sampling country	Gouda	Brie	Total
Total	19 (1)	15 (1)	34 (2)
France	-	3 (-)	3 (-)
Germany	8 (-) [(a)]	1 (-) [(b)]	9 (-)
Greece	-	3 (-)	3 (-)
Italy	-	3 (-)	3 (-)
Netherlands	8 (1)	3 (1)	11 (2)
Spain	3 (-)	2 (-)	5 (-)

(a): Including 4 samples of Emmental cheese.
(b): Including 1 sample of Camembert cheese.

Infant formula. In Table 27 the samples collected of infant formula are shown. Infant formulas were subdivided into three categories depending of the age of the infant (new-born, and two follow-up formulas).

Table 27: Infant formula samples collected per country and type. The number of samples of organic production is given between brackets.

Sampling country	Recommended age (months)			Total
	0-6	6-10/12	10/12-36	
Total	8 (2)	10 (1)	7 (-)	25 (3)
France	-	1 (-)	2 (-)	3 (-)
Germany	2 (1)	2 (-)	1 (-)	5 (1)
Greece	2 (-)	-	-	2 (-)
Italy	1 (-)	2 (-)	-	3 (-)
Netherlands	2 (1)	3 (-)	2 (-)	7 (1)
Spain	1 (-)	2 (1)	2 (-)	5 (1)

13.1.2. Eggs and egg products

A total of 205 egg samples were collected during the course of the survey (Table 28). The sampling of eggs covered the four main types of production (indicated by a production code on the eggs), being either cage ('0'), barn ('1'), free range ('2') or organic ('3') eggs. Sampling was based on their availability in the specific food retail market. For instance, in the Netherlands and Germany most of the eggs are free range or barn, while in other countries eggs may still be produced by laying hens kept in cages.

The sampling of 'Egg products' as specific category was discarded, due to the fact that dried egg products (e.g. complete egg, egg white, egg yolk) were not available at most retail shops in the countries of sampling.

Table 28: Egg samples collected per country and type

Sampling country	Cage	Indoor (barn)	Free range	Organic [a]	Unknown [b]	Total
Total	40	65	62	30	8	205
France	12	-	11	2	1 [c]	26
Germany	-	18	17	5	-	40
Greece	12	4	-	2	2	20
Italy	6	14	2	3	-	25
Netherlands	-	22	19	12	-	53
Spain	10	7	13	6	5	41

(a): Organic eggs are produced by laying hens under free range conditions.
(b): Type of production not indicated on the label (presumably these samples are from cage production).
(c): One sample of fresh quail eggs was collected.

13.1.3. Meat and meat products

In the original plan (Table 2) it was foreseen to collect 300 meat and liver products during the course of the survey. Since in none of the 150 meat samples collected and analysed during the first sampling round PAs were detected, for the second sampling round it was decided to reduce the total number of meat and liver samples to 250. In Table 29 the number of samples collected per country is presented. A total of 242 meat samples were collected, 36 of them being from organic production (15 %). Approximately equal number of samples of beef, pork and poultry meat were collected. The sampling focused on a type of cut instead of diversification of products. Thus, for poultry chicken breast fillets and for pork loin filets were collected. The scenario differed a bit regarding beef meat since cuts may differ among countries and are not always available on the market. Due to considerations such as price and low-fat content, it was decided to sample any cut of the round area of the bovine.

Liver samples were purchased and analysed during the second sampling period. It was anticipated that the liver matrix could very unstable due to enzymatic reactions, therefore the samples were stored at -80 °C as soon as they were purchased. Care was taken that products were purchased with expiration dates indicating that the product was still very fresh at the moment of purchase. Due to a somewhat limited availability of liver products in supermarkets and retail shops in the countries sampled, part of the liver samples were bought at specialised butcher shops. Sampling was limited to the three countries of the partners of the consortium (the Netherlands, Germany and Spain) to minimise as much as possible transport and consequently degradation. Overall, equal numbers of beef, pork and poultry liver were collected during the second sampling round (Table 29). Consumption habits differed between the three countries, beef and pork liver being more common in Germany and Spain, while chicken liver is more popular in the Netherlands.

Table 29: Meat and liver samples collected per country and type. The number of samples of organic production is given between brackets.

Sampling country	Beef meat	Pork meat	Chicken meat	Beef liver	Pork liver	Chicken liver	Total
Total	80 (14)	79 (10)	83 (12)	11 (1)	10 (-)	10 (1)	273 (38)
France	10 (2)	10 (1)	10 (2)	-	-	-	30 (5)
Germany	14 (2)	15 (2)	18[a] (3)	5 (-)	4 (-)	1 [b] (-)	57 (7)
Greece	8 (2)	8 (2)	8 (1)	-	-	-	124 (5)
Italy	10 (1)	10 (-)	10 (1)	-	-	-	30 (2)
Netherlands	15 (3)	14 (2)	14 (2)	-	2 (-)	9 (1)	54 (8)
Spain	23 (4)	22 (3)	23 (3)	6 (1)	4 (-)	-	78 (11)

(a): Including 4 samples of turkey filet.
(b): Including 1 sample of turkey liver.

13.2. Plant-derived food products

A summary of the sampling plan for plant-derived products and the number of products collected is shown in Table 30. The majority of samples were purchased from food retails. In total 359 samples were purchased (20 % more than the number originally proposed at the start of the project, see Table 2). For both major food categories a higher number of samples were collected than proposed. A total of 61 samples of organic production were collected, corresponding to 17 % of the total, while at the start of the project only 40 samples of organic production (13 %) were foreseen. The percentage of organic samples was highest in the category of (herbal) teas, but also organic herbal food supplements were available in retail shops.

Table 30: Overview of plant-derived food samples collected and analysed

	Target number of samples	Samples collected and analysed	Organic samples	% Organic
All teas and food supplements	**300**	**359**	**61**	**17 %**
Teas	**150**	**168**	**33**	**20 %**
Black tea	30	33	4	12 %
Green tea	20	26	4	15 %
Rooibos tea	20	22	7	32 %
Chamomile tea	30	35	7	20 %
Peppermint/poleo mint tea	30	30	6	20 %
Mixed herbal tea	20	22 [(a)]	5	23 %
Food supplements	**150**	**191**	**28**	**12 %**
Supplements based on plants not known to produce PAs	75	111	12	11 %
Supplements based on plants known to produce PA**s**	50	51	11	22 %
Supplements containing bee products	25	29	5	17 %

(a): Including 2 samples of fennel tea.

Figure 14 shows an overview of the number of plant-derived products collected over the course of the survey. Samples were collected from January 2014 till February 2015 in two major sampling rounds. During the first sampling period (January to April 2014) all 168 (herbal) teas were collected and during the second sampling period (August 2014 to February 2015) all 191 food supplements. The majority of products had a long shelf-life (1-2 years), thus the time of sampling does not reflect the time of production.

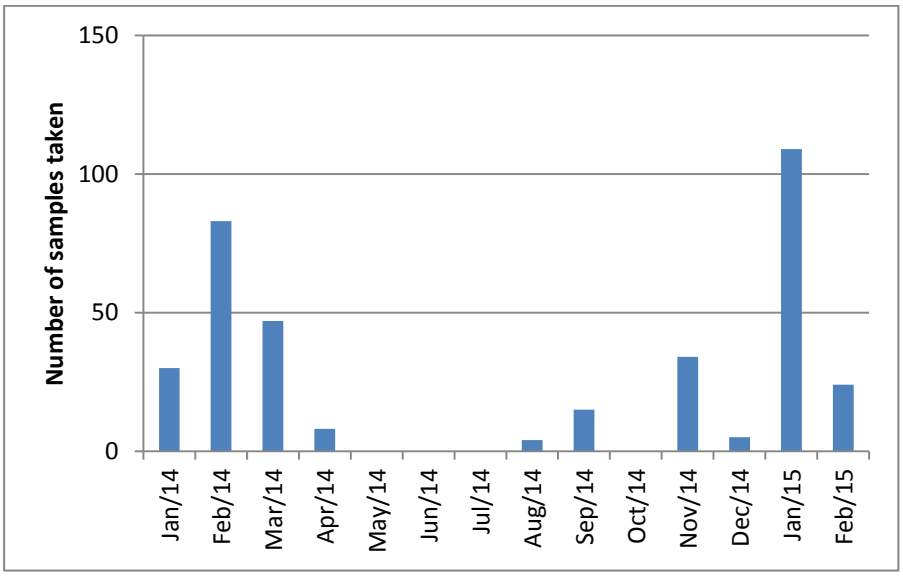

Figure 14: Overview of the monthly number of products collected of plant origin

An overview of the country of production of the plant-derived food samples collected is presented in Table 31. For more than 60 % of the samples the country of production is not indicated. This is particularly the case for the (herbal) teas. It may be anticipated however that most of the black and green teas originate from Asia, and rooibos tea is typically produced in South Africa. The country of production is more often indicated on the food supplement labels. Germany, United Kingdom and the USA appear to be the main producers of these supplements. From Table 31 it is evident that products containing plant-derived material, sold in European retail shops, can come from around the world.

Table 31: Overview of plant-derived food samples collected and analysed

Country of production	(Herbal) teas	Food supplements	Total	% of total
Total	168	191	359	
Asia	3	-	3	0.8 %
Canada	-	1	1	0.3 %
China	6	-	6	1.7 %
EU	-	7	7	1.9 %
France	1	1	2	0.6 %
Germany	1	24	25	7.0 %
Greece	-	1	1	0.3 %
India	4	1	5	1.4 %
Netherlands	-	3	3	0.8 %
South Africa	10	1	11	3.1 %
South Korea	-	1	1	0.3 %
Spain	-	9	9	2.5 %
Sri Lanka	4	-	4	1.1 %
Thailand	-	1	1	0.3 %
United Kingdom	-	20	20	5.6 %
USA	-	29	29	8.1 %
Vietnam	1	-	1	0.3 %
Unknown	138	92	230	64.1 %

13.2.1. (Herbal) teas

The sampling of the tea products was conducted during the first sampling period. Both tea bags and loose tea were sampled. In total 168 samples were collected from 6 different types of tea (Table 32). According to the UK Tea association[4] 90 % of tea is sold as tea bags. In Germany the situation may be different for fermented tea since 60 % of the black tea is sold as loose tea and 40 % as tea bags. In this survey, 78 % of the samples were tea bags and 22 % were collected as loose tea. According to the German Tea Association[5] only 2-3 % of tea is sold as organic tea. According to the original EFSA project proposal, 13 % of the tea samples should be from organic production. In this survey 20 % of the samples collected were from organic origin. Organic tea was readily available in retail shops in all countries samples except Greece.

Table 32: Tea samples collected per country and type. Number of samples of organic production between brackets.

Sampling country	Black		Green		Rooibos		Chamomile		Peppermint		Mixed		Total	
	Bag	Loose	Bag	Loose	Bag	Loose	Bag	Loose	Bag	Loose	Bag	Loose	Bag	Loose
Total	24 (2)	9 (2)	19 (3)	7 (1)	18 (5)	4 (2)	28 (5)	7 (2)	24 (4)	6 (2)	17 (3)	5 (2)	130 (22)	38 (11)
France	2 (-)	2 (1)	1 (-)	1 (-)	1 (-)	-	3 (-)	1 (1)	2 (1)	1 (1)	-	1 (-)	9 (1)	7 (3)
Germany	10 (1)	3 (-)	7 (-)	1 (-)	7 (-)	2 (1)	11 (2)	1 (-)	11 (-)	1 (-)	7 (1)	1 (-)	53 (4)	9 (1)
Greece	1 (-)	1 (-)	1 (-)	-	-	-	1 (-)	1 (-)	1 (-)	1 (-)	1 (-)	-	5 (-)	3 (-)
Italy	1 (-)	1 (1)	2 (1)	1 (-)	1 (1)	1 (1)	3 (1)	2 (1)	1 (-)	1 (1)	3 (1)[(a)]	1 (1)[(a)]	12 (4)	8 (5)
Netherlands	5 (-)	1 (-)	4 (1)	1 (1)	5 (3)	-	5 (1)	1 (-)	4 (2)	1 (-)	1 (-)	2 (1)	25 (7)	6 (2)
Spain	4 (1)	1 (-)	4 (1)	1 (-)	4 (1)	1 (-)	5 (1)	1 (-)	5 (1)	1 (-)	4 (1)	-	26 (6)	5 (-)

(a): Including 1 sample of fennel tea.

13.2.2. Herbal food supplements and bee products

The sampling of herbal food supplements focused on those based on (i) ingredients not known to produce PAs, (ii) ingredients known to produce PAs and (iii) ingredients containing bee products.

Herbal food supplement samples were purchased in pharmacies, drugstores, supermarkets, herbalist shops and via webshops. Herbal food supplements are often sold in the form of tablets or capsules that contain either dried plant material, (dried) plant extracts or the essential oil of the plant. Sometimes the herbal supplement is presented in the form of an (alcoholic) extract of the plant (mixture). Some herbal food supplements are sold as dried, loose, plant material to be used as herbal tea infusion. Products that could be classified as Traditional Chinese Medicines (TCM) were not included in this survey.

The labels of the food supplements were checked for any information on the different plant ingredients included in the herbal preparation. If there were any ingredients listed that could be linked to known PA-producing plants, the food supplement was listed as such. Ingredients known to produce PAs are for instance *Borago*, *Echium*, *Eupatorium*, *Lithospermum*, *Petasitis*, *Pulmonaria*, *Senecio*, *Symphytum* and *Tussilago* species (Tables 33 and 38). Some PA-producing plants are used as pharmaceuticals, as for instance species of *Echium*, *Borago*, *Symphytum*, *Petasitis* and *Tussilago*. Some of them are also sold as food supplements. In Germany species of *Echium* and *Borago* are available. This situation may differ in other European countries, where extracts of *Echium* and *Borago* are mostly sold as oil-based supplements. Therefore, for each sample it was checked that it was not labelled as a pharmaceutical product.

[4] http://www.tea.co.uk/tea_directory
[5] www.teaverband.de/english

Table 33: Samples of herbal food supplements collected per type and country. The number of samples from organic production is given between brackets.

Sampling country	Supplements based on plants not known to produce PAs	Supplements based on plants known to produce PAs	Supplements containing bee products	Total
Total	111 (12)	51 (11)	29 (5)	191 (28)
France	10 (1)	5 (-)	3 (-)	18 (1)
Germany	43 (4)	10 (4)	11 (3)	64 (11)
Greece	7 (1)	4 (-)	1 (-)	12 (1)
Italy	9 (2)	3 (1)	2 (-)	14 (3)
Netherlands	22 (-)	10 (2)	5 (-)	37 (2)
Spain	20 (4)	19 (4)	7 (2)	46 (10)

14. OCCURRENCE OF PYRROLIZIDINE ALKALOIDS IN FOOD PRODUCTS

14.1. Occurrence of PAs in animal-derived food products

In total 746 products from animal origin were analysed for the presence of 35 different PAs. PAs were detected above the LOD in a number of milk and egg samples, but no positive findings were recorded for yoghurt, cheese, infant formula, meat and liver samples. The positive findings are summarised in Table 34. Milk samples containing one or more PAs above the LOD (0.03-0.05 µg/L) in the first analysis were reanalysed using a different subsample to confirm the finding. The same approach was used when egg samples contained one or more PAs above the LOD (0.05-0.15 µg/kg) in the first analysis. When the presence of a PA was confirmed in the second sample, the average content of the two samples is reported.

Table 34: Milk and egg samples containing one or more PAs above the LOD. Average concentration of two independent analytical measurements, concentration in µg/L (milk) or in µg/kg (eggs).

Sample	Origin	Description	Organic/ Non-organic	Pyrrolizidine alkaloid	Conc. (µg/L in milk; µg/kg in eggs)
FB14/0204	Germany	Semi-skimmed milk, past.	Non-organic	Senkirkine	0.05
FB14/0210	Germany	Skimmed milk, past.	Non-organic	Otosenine	0.08
FB14/0211	Germany	Semi-skimmed milk, past.	Organic	Otosenine	0.06
FB14/0235	Germany	Semi-skimmed milk, past.	Organic	Otosenine	0.11
IRTA 510	Greece	Skimmed milk, UHT	Non-organic	Senkirkine	0.16
IRTA 514	Greece	Whole milk, UHT	Non-organic	Senkirkine	0.06
RIK M20	Netherlands	Whole milk, pasteurised	Non-organic	Lycopsamine	0.12
RIK M21	Netherlands	Semi-skimmed milk, past.	Organic	Jacoline	0.05
IRTA 153	Spain	Semi-skimmed milk, UHT	Organic	Jacoline	0.06
IRTA 639	Spain	Whole milk, pasteurised	Organic	Lycopsamine Echimidine	0.11 0.06
IRTA 652	Spain	Goat milk, UHT	Non-organic	Retrorsine	0.11
FB14/0111	Germany	Free range eggs	Non-organic	Lycopsamine	0.12
FB14/0138	Germany	Barn eggs	Non-organic	Retrorsine	0.10

As shown in Table 34, in 11 out of 182 (6.0 %) milk samples the presence of one or two PAs could be confirmed above the LOD. PAs were more often found in samples from organic production (5 out of

44, 11 %) than from regular production (6 out of 132, 4.3 %), but the number of samples is too small to draw conclusions on this point. PA residues were found in milk from 4 different countries (Spain, Germany, Greece, the Netherlands) and in all major types of milk regarding fat content and process of conservation. Six different PAs were found, representing the macrocyclic senecionine-type (jacoline, retrorsine), otonecine-type (senkirkine, otosenine) and open chain retronecine type (lycopsamine, echimidine). Only the free base form of PAs were found and no PANOs were detected, which is in accordance with the PA transfer study conducted by Hoogenboom et al. (2011) and which may be due to the role of the rumen in digesting the plant material, whereby the PANOs are degraded or converted to PA FBs. Interestingly, in this transfer study it was reported that for three of the PAs detected in this survey (jacoline, senkirkine and otosenine) the carry-over rate was relatively high (Hoogenboom et al., 2011).

Contamination of eggs with PAs was only found in two samples out of 205 analysed (1 %). Levels are very low (0.1-0.12 µg/kg). The PAs found are similar to the ones found in milk, and again only in the free base form. Transfer of PAs from feed to eggs has been shown to occur (Edgar et al., 2000; Eröksüz et al., 2003; Diaz et al., 2014). Diaz et al. (2014) reported that the residues found in eggs were primarily of the PA free base type with only a very minor contribution of PANOs.

As discussed in Section 11.1, it could not be *a priori* excluded that egg and meat samples contained (traces of) PAs at the moment of production or slaughter. Other than for milk, where the microbiological action is effectively stopped or strongly reduced during processing, possibly microbiological (and enzymatic) conversions continue to take place in eggs and meat under the normal storage conditions in retail shops and supermarkets. A model experiment using different types of meat spiked with PAs, did not show any significant breakdown or conversion of PAs during short-term storage at 4-6 °C (Section 11.1.3). Furthermore, in a recently conducted PA transfer study with laying hens, no change in PA composition in the contaminated eggs was observed after storage of up to 8 weeks at room temperature and at 4-6 °C (RIKILT, unpublished results). The test experiments with eggs and meat thus indicate that breakdown of PAs under the typical conditions of storage is not a major issue.

From the results obtained for the animal-derived food products it can be concluded that:

- Contamination of eggs and meat products with PAs seems to be very rare in the European Union.
- With model experiments it could be shown that it is not likely that microbiological degradation of PAs in meat products during storage under retail conditions is a major issue.
- PAs are occasionally found in milk samples. Concentrations in milk are low (in the sub-µg/L range). This may not be that surprising as generally milk is mixed to a large extent during processing, whereby the contaminants are effectively diluted, but at the same time also spread to a much greater extent. This may thus offer an explanation why PAs are more often found in milk than in other animal-derived products such as eggs and meat.
- The type of PAs found in milk suggests that plant material of *Senecio* and *Boraginaceae* spp may be the cause of contamination.

14.2. Occurrence of PAs in plant-derived food products

14.2.1. Occurrence of PAs in (herbal) teas

A summary of the results obtained is given in Table 35. The average, median, as well as 75th and 95th percentiles for the total sum of PAs, the sum of PA free bases and the sum of PANOs for the different types of tea infusions is given in Appendix F. The same parameters expressed as content of dry tea is given in Appendix G. Data on individual PA obtained for positive samples are also reported in Appendix H. For fennel tea, the two samples analysed did not contain any PAs above the LOD. As the number of samples was too low for any statistical evaluation, results for fennel teas were not included in any further evaluation according to the different types of tea.

Table 35: Types of tea infusions analysed for PAs [(a)]. Total PA levels measured in different types of tea (sum of 28 individual PAs), expressed as tea infusion and as dry tea.

Type of tea	N	% of samples > LOD	Tea infusion (µg/L)				Dry tea (µg/kg)			
			Min.	Max.	Mean	Median	Min.	Max.	Mean	Median
All teas	**166**	**91.0**	**<LOD**	**64.08**	**6.13**	**2.47**	**<LOD**	4 804.5	459.6	184.7
Black	33	93.9	<LOD	54.16	7.62	1.59	<LOD	4 061.5	571.6	118.6
Chamomile	35	85.7	<LOD	18.59	3.65	1.69	<LOD	1 394.3	273.8	124.7
Green	26	85.2	<LOD	52.22	5.65	0.33	<LOD	3 916.6	423.4	24.5
Mixed herbs	20	95.2	<LOD	25.72	5.82	2.47	<LOD	1 929.2	439.4	180.2
Peppermint	30	93.1	<LOD	58.69	6.68	2.60	<LOD	4 401.0	496.2	195.6
Rooibos	22	95.5	<LOD	64.08	7.99	3.26	<LOD	4 804.5	598.5	244.0

(a): Excluding 2 samples of fennel tea.

The results of Table 35 show that contamination of all types of tea with PAs is very common. In the majority of samples (91 %) one or more PA was detected. All types of teas appear to contain PAs, although the concentrations differed between the various types of tea. Highest contamination, with regard to maximum, mean and median concentration, was observed in rooibos tea, while green tea showed the lowest median concentration, chamomile tea the lowest maximum and mean concentration.

The wide range of detected concentrations is explicitly demonstrated in Figure 15, where the distribution of PA concentrations of individual samples is shown with regard to the type of tea. The PA content ranged widely as almost each tea type contained samples below the LOD but also concentrations in the µg/L range.

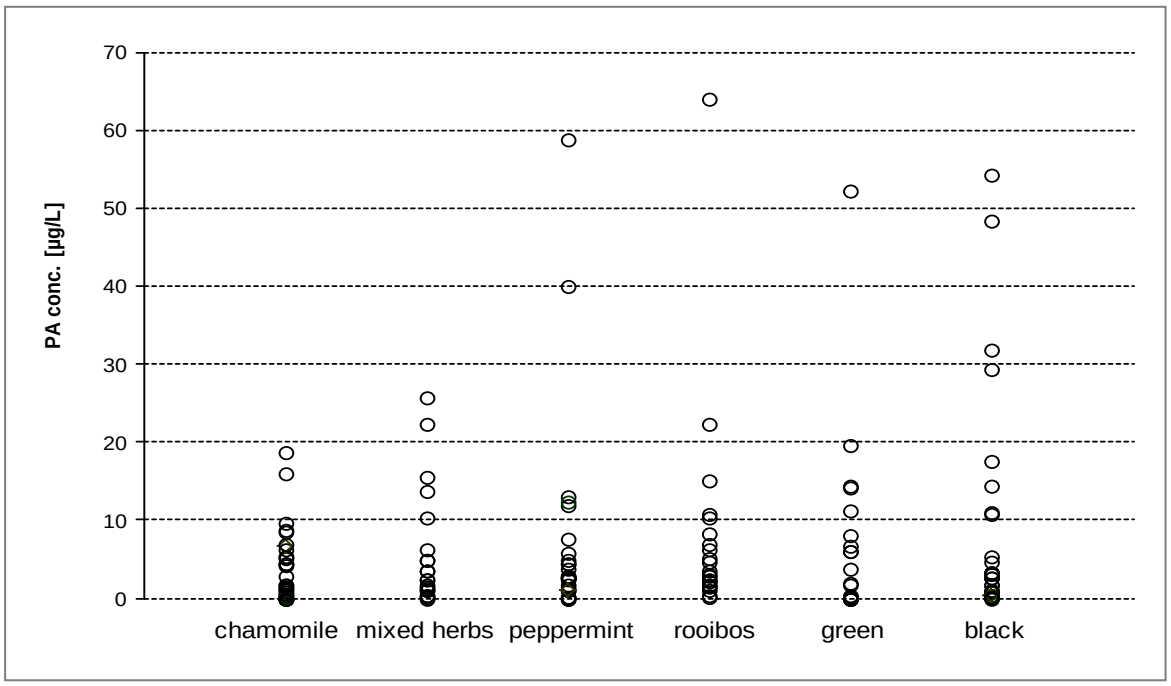

Figure 15: Total PA concentrations (µg/L) determined in infusions prepared from various types of tea

The results were evaluated with regard to the form of production, as approximately 20 % of the purchased teas were derived from organic production. Further, it was of interest whether the form of packaging (loose or bag) might have any correlation to the PA content. Results of mean PA contents for organic and non-organic teas and type of packing are shown in Figure 16.

The results indicate that teas from organic production tend to contain lower PA concentrations. This seems also be the case for the PA content in loose teas compared to teas in bags.

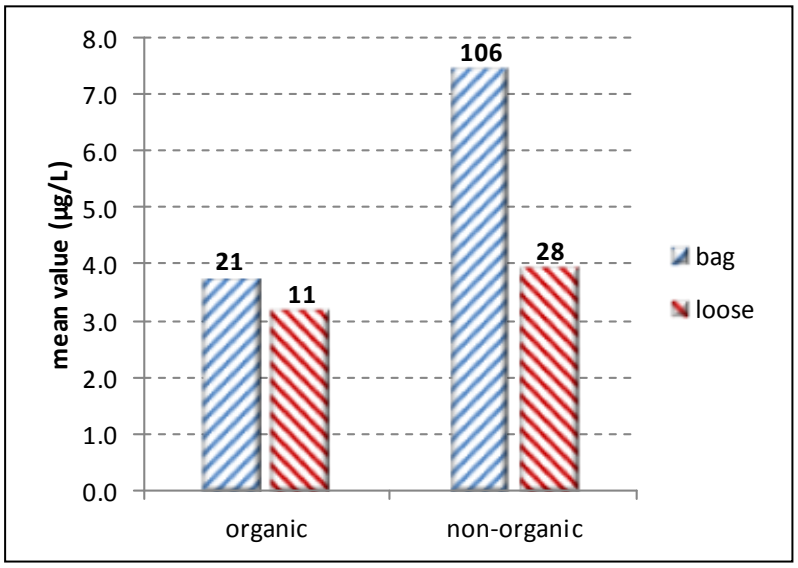

Figure 16: PA mean concentrations (µg/L) in relation to the type of production (organic, non-organic) and type of packing (bag, loose)

A more detailed representation is given in Figure 17 where all results of the investigated types of tea have been categorized according to organic and non-organic production and to the packing form. For instance, the mean concentration for the sum of PAs in black and green tea from non-organic production is 7.72 µg/L (n = 51) while for the products from organic production is 0.61 µg/L (n = 7). The same trend is found for peppermint and mixed herbal teas. Organic teas (green shapes in Figure 17) tend to have lower PA contents compared to non-organic teas (blue shapes in Figure 17). Further, loose teas (circles in Figure 17) tend to have lower PA concentrations compared to teas in bags (triangles in Figure 17).

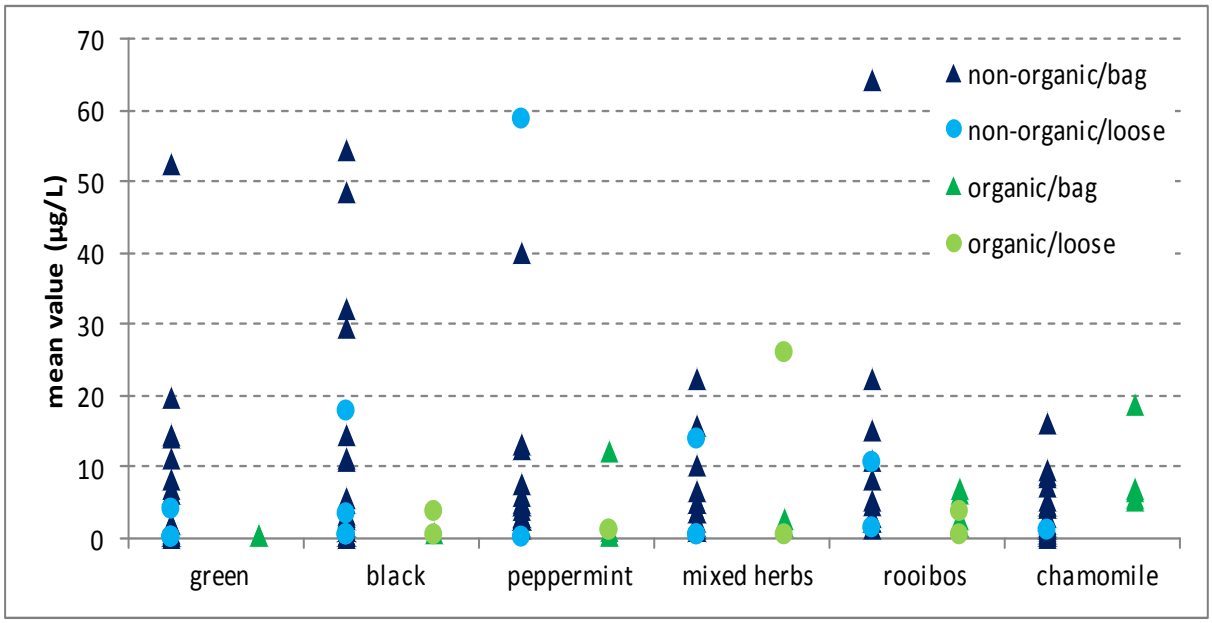

Figure 17: Concentration of the sum of PAs in different types of tea subdivided according to the form of production and form of packing

Tea samples were further evaluated concerning their content of individual PAs according to: (i) the frequency of occurrence, i.e. in which percentage of the samples particular PAs were detected and (ii) the respective mean concentration of a particular PA in all samples. Figure 18 shows the results for all (herbal) tea samples combined. Overall, senecionine-type PAs (such as retrorsine, senecionine, seneciphylline, senecivernine and the corresponding PANOs) are the most prominent PAs found in tea, with individual occurrence percentages between 40 and 65 %. Intermedine is also often present in tea. With respect to contribution to the mean content in tea infusions, senecionine-*N*-oxide is the most important compound with an average concencentration of 1.74 µg/L, which makes up 28 % of the total PA concentration (6.13 µg/L) found in tea. The PAs of the senecionine group account for over 77 % of the PA content in tea, while PAs of the lycopsamine group contribute 14 %, and heliotrine-type PAs contribute 8 %. PAs from the monocrotaline group were not detected in any of the tea samples. Approximately one third of the content of PAs in the tea samples is made up by PA free bases and two-thirds by PANOs.

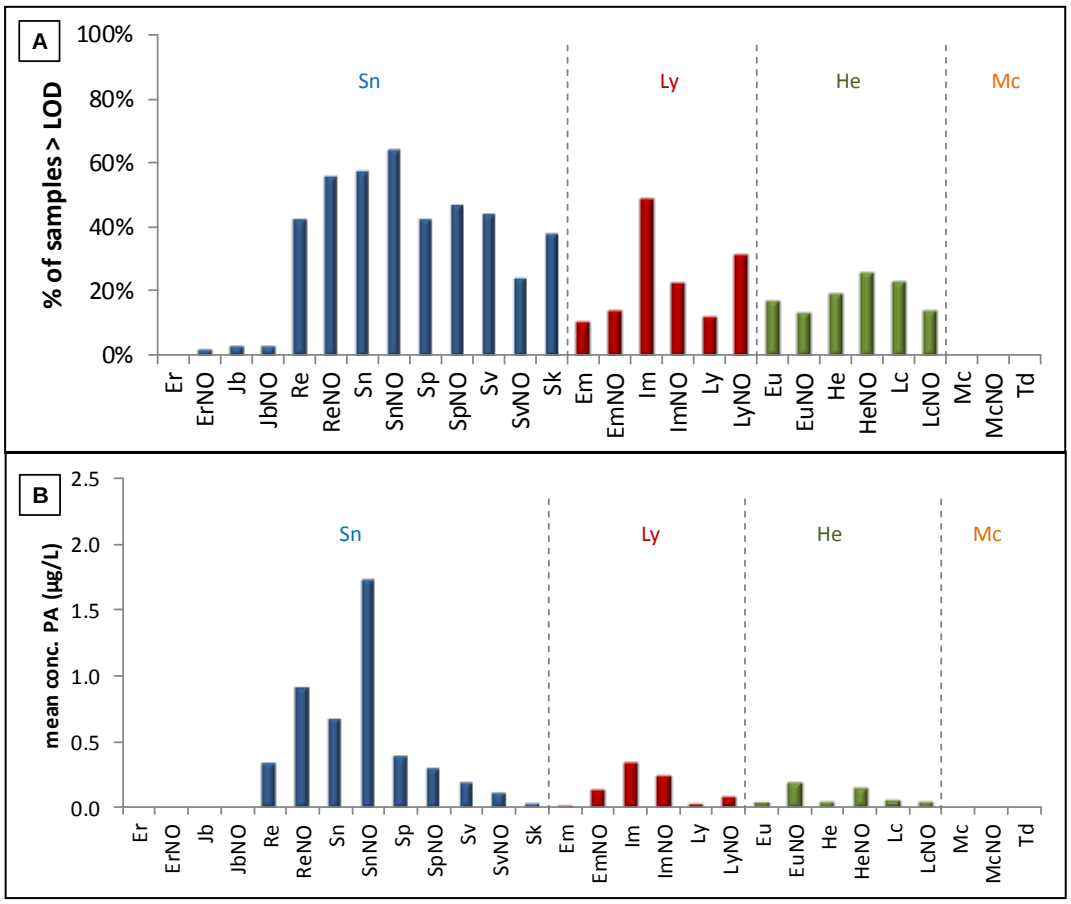

Figure 18: (A) Percentage of 166 tea samples containing an individual PA > LOD and (B) mean concentrations of individual PAs in the investigated tea samples. Both graphs are separated into four categories – summarising senecionine-type (Sn), lycopsamine-type (Ly), heliotrine-type (He) and monocrotaline-type (Mc) PAs, respectively.

However, there are important differences in the individual PA composition and concentration found in the different types of tea. The results for the main types of are discussed below.

In Figure 19 the distribution of PAs in black and green teas is shown. The most frequently occurring PAs belong to the senecionine- and lycopsamine-type, while the heliotrine-type is practically absent. The mean concentrations are dominated by retrorsine, senecionine and intermedine and their respective *N*-oxides, together contributing for over 95 % of the total PA content. Black and green teas appear to be contaminated with plant material containing a relatively simple PA profile, with only three PAs that dominate. Of these PAs, senecionine and retrorsine are produced in high concentrations in the *Senecio* species, while intermedine is a representative for species belonging to the *Boraginaceae* family, that includes genera such as *Anchusa*, *Borago*, *Symphytum* and *Echium*, but it has also been detected in *Eupatorium* species (family of *Asteraceae*) (Hartmann and Witte, 1995; El-Shazly and Wink, 2014).

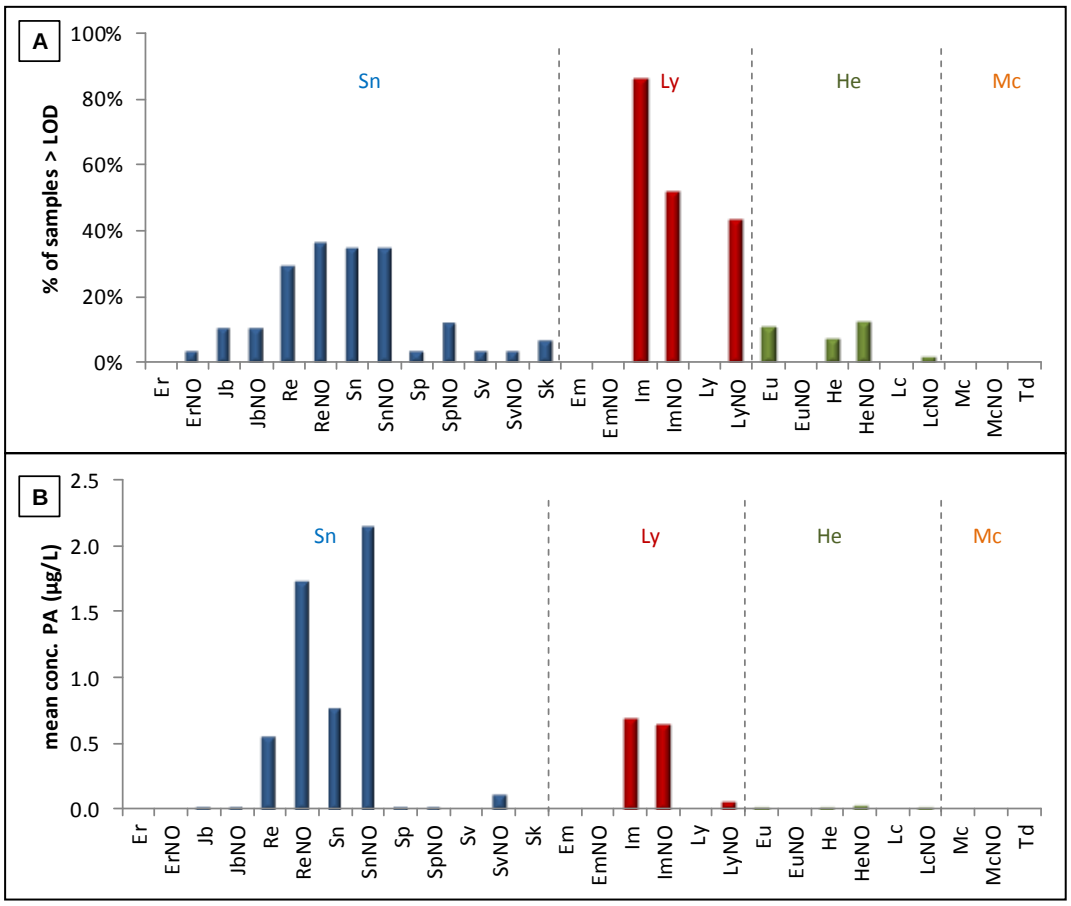

Figure 19: (A) Percentage of black and green tea samples (n = 59) containing at least one individual PA > LOD, and (A) mean concentrations of individual PAs in the investigated tea samples. Both graphs are separated into four categories – summarising senecionine-type (Sn), lycopsamine-type (Ly), heliotrine-type (He) and monocrotaline-type (Mc) PAs, respectively.

In Figure 20 the distribution of PAs in mixed herbal teas is shown. In this sample group, PAs of senecionine-type and heliotrine-type are found most frequently and at the highest conentration, while PAs of the lycopsamine-type occur with lower abundance. Senecionine-*N*-oxide, europine-*N*-oxide and heliotrine-*N*-oxide are the three main PAs found in this type of tea. Together they account for almost 40 % of the PA content. Senecionine-type and heliotrine-type PAs account for over 90 % of the PA content in mixed herbal teas. This PA pattern indicates that species of *Senecio* and *Heliotropium* are the most relevant contaminating species during production of mixed herbal teas. The mean concentrations of individual PAs are relatively low compared to other investigated teas (except chamomile). The PA pattern is more complex than found in e.g. green and black teas. This is expected as mixed herbal teas are typically blended teas with a larger variety of ingredients, that may come with different types of PA contamination.

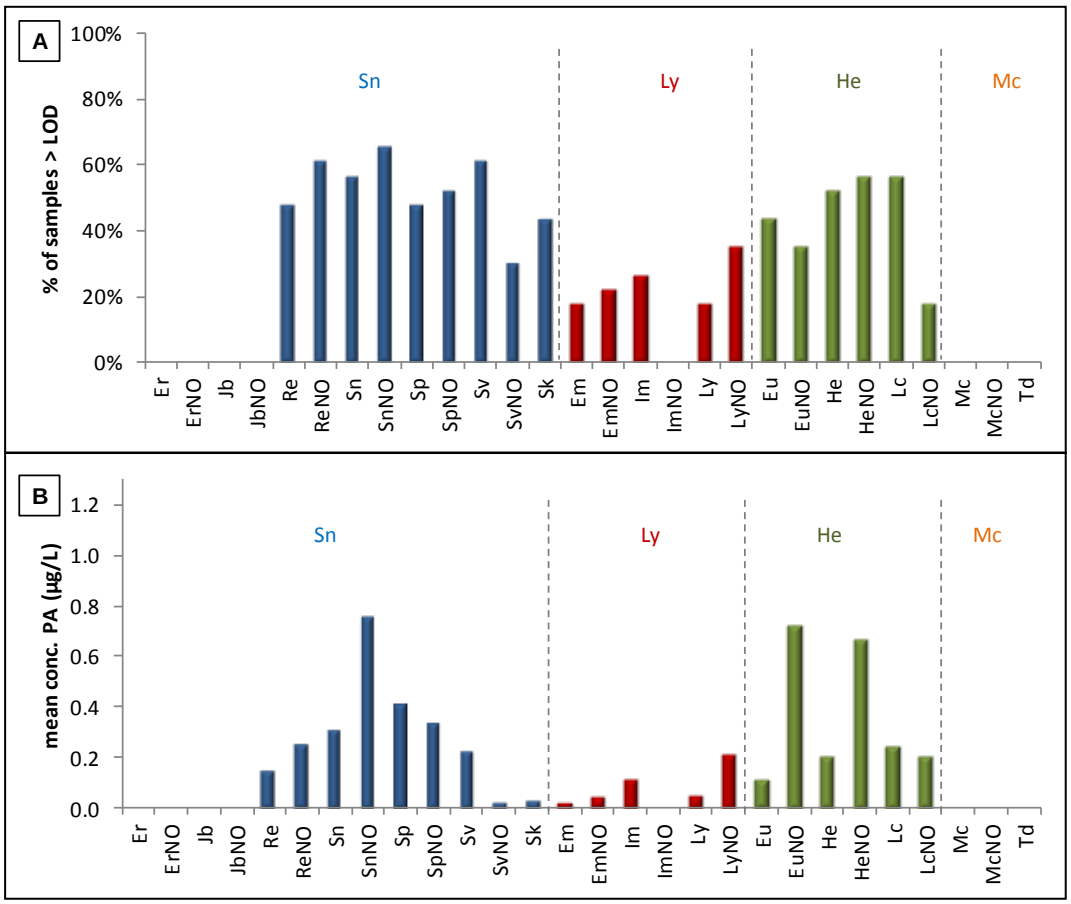

Figure 20: (A) Percentage of 23 mixed herbal tea samples containing an individual PA > LOD and (B) mean concentrations of individual PAs in the investigated tea samples. Both graphs are separated into four categories – summarising senecionine-type (Sn), lycopsamine-type (Ly), heliotrine-type (He) and monocrotaline-type (Mc) PAs, respectively.

In Figure 21 the distribution of PAs in chamomile teas is shown. PAs of the senecionine-type are found the most often, followed by lycopsamine-type PAs. The mean concentrations of individual PA are relatively low compared to other types of investigated teas (except mixed herbs). The PAs detected with the highest mean concentrations were senecionine, intermedine and the *N*-oxides of senecionine, echimidine and europine. The general PA pattern observed in chamomile teas suggests that species of *Senecio*, *Boraginaceae* and *Heliotropium* may all be relevant contaminating species in this type of tea.

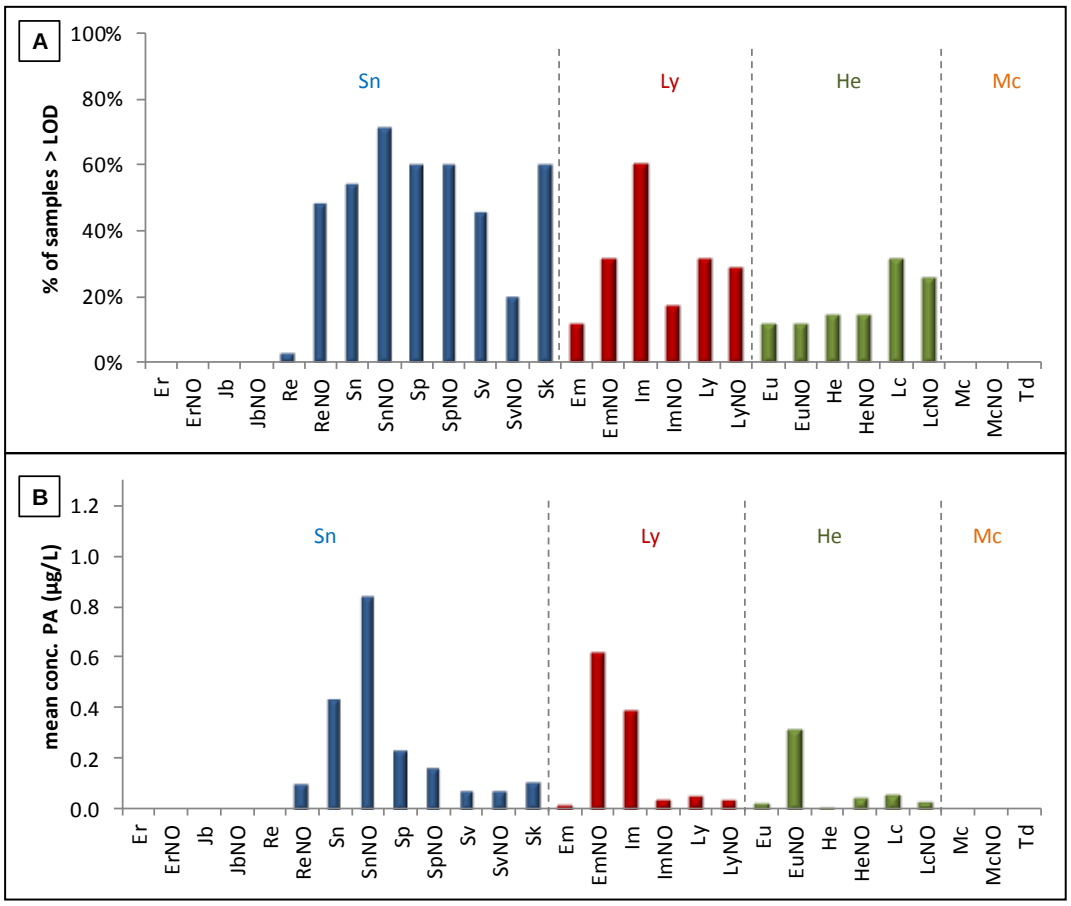

Figure 21: (A) Percentage of 35 chamomile tea samples containing an individual PA > LOD and (B) mean concentrations of individual PAs in the investigated tea samples. Both graphs are separated into four categories – summarising senecionine-type (Sn), lycopsamine-type (Ly), heliotrine-type (He) and monocrotaline-type (Mc) PAs, respectively.

In Figure 22 the distribution of PAs in peppermint teas is shown. For this type of tea mostly PAs of the senecionine-type were detected, with smaller contributions of heliotrine and lycopsamine-type PAs. Compared to the normally observed distribution of senecionine-type PAs, where senecionine and retrorsine are dominating, in peppermint teas seneciphylline was detected in a relatively high concentration and abundance. Seneciphylline, seneciphylline-*N*-oxide and senecionine-*N*-oxide together account for approximately 50 % of the total PA content in peppermint teas.

Plants of *Senecio* species seem to be the most important with respect to contamination of peppermint teas, although *Heliotropium* and species of the *Boraginaceae* family, may contribute to a smaller amount as well. The PA pattern found for the senecionine-type PAs correlates particularly well to that of *Senecio vulgaris* (common groundsel) (Mulder et al., 2009; de Nijs et al., 2014).

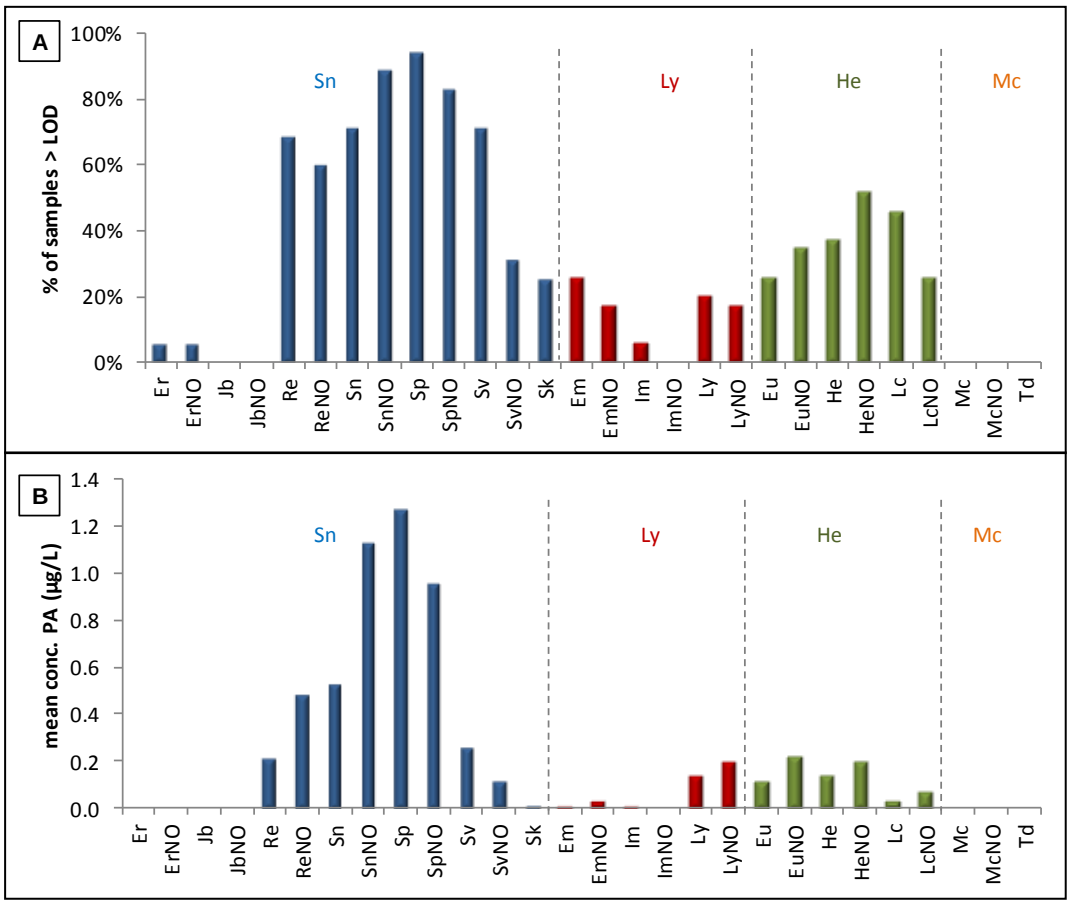

Figure 22: (A) Percentage of 35 peppermint tea samples containing an individual PA > LOD and (B) mean concentrations of individual PAs in the investigated tea samples. Both graphs are separated into four categories – summarising senecionine-type (Sn), lycopsamine-type (Ly), heliotrine-type (He) and monocrotaline-type (Mc) PAs, respectively.

In Figure 23 the distribution of PAs in rooibos teas is shown. In these teas the PA pattern is quite different compared to other teas. The PA profile practically only contains senecionine-type PAs. Almost all samples contained retrosine, senecionine, senecivernine and their *N*-oxides as well as low levels of senkirkine. The PA pattern comes closest to that found in green and black tea. The PA mean concentrations are higher than in any other type of tea. The contribution is dominated by senecionine-N-oxide, as a single PA making up almost 50 % of the mean PA content. Together the senecionine-type PAs are responsible for 98 % of the PA content in rooibos tea. The type of PAs found in rooibos tea strongly point to contamination with *Senecio* spp. Rooibos tea is typically produced in South Africa, which is known for its presence of many toxic *Senecio* species (Stewart and Steenkamp, 2001).

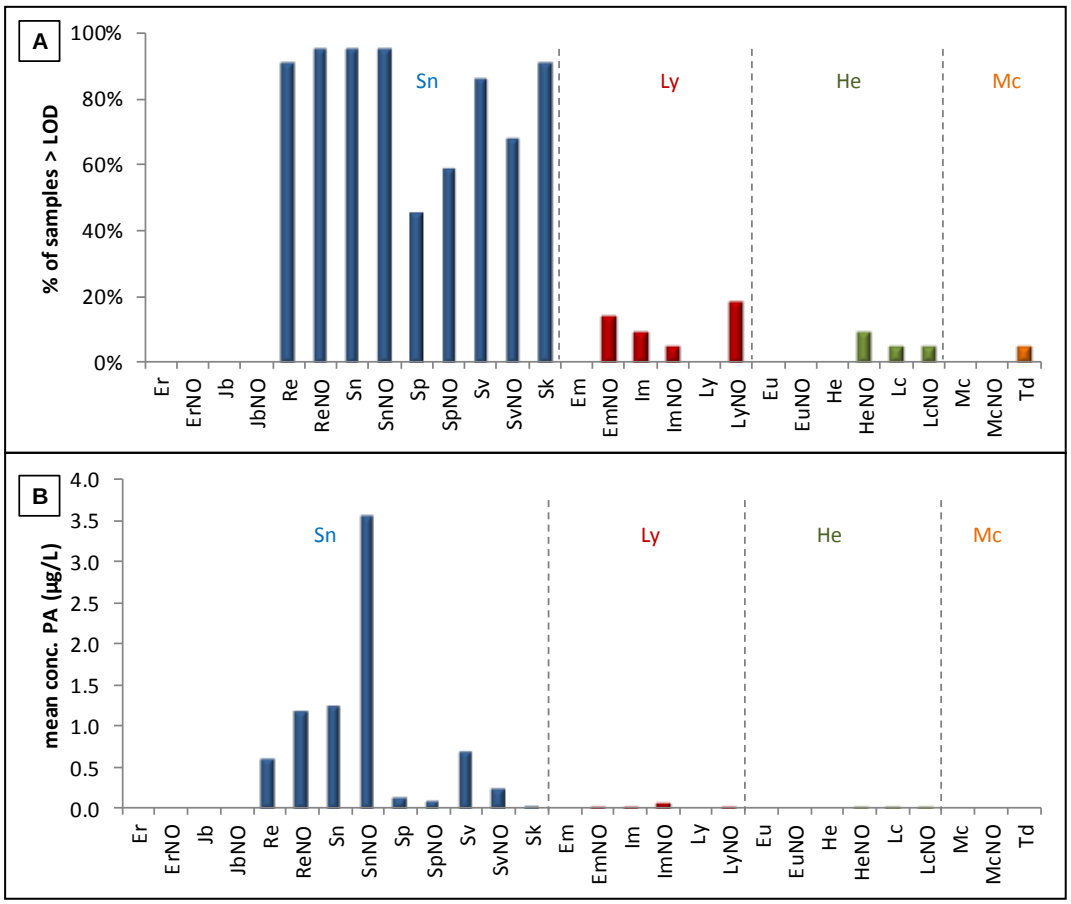

Figure 23: (A) Percentage of 22 rooibos tea samples containing an individual PA > LOD and (B) mean concentrations of individual PAs in the investigated tea samples. Both graphs are separated into four categories – summarising senecionine-type (Sn), lycopsamine-type (Ly), heliotrine-type (He) and monocrotaline-type (Mc) PAs, respectively.

The results obtained in this survey for (herbal) teas correlate well with the results reported from other recent studies (BfR, 2013; Bodi et al., 2014; Mathon et al., 2014; Griffin et al., 2014; Schulz et al., 2015). The most important results of these recent studies will be briefly discussed below.

Bodi et al. (2014) analysed a total of 274 dry tea samples available on the German market, including, amongst others, 24 black, 23 green, 24 rooibos, 29 peppermint, 39 chamomile and 43 mixed herbal teas, for the presence of 10 different PA FBs and 7 different PANOs with LC-MS/MS. LODs reported were in the range of 0.5-2 µg/kg, comparable to the LODs in this study (see Table 16). The percentage of positive teas varied between 86 % (peppermint teas) to 100 % (rooibos teas). As in this study, rooibos tea was found to be the most highly contaminated (mean: 1 856.4 µg/kg, maximum: 5 647.2 µg/kg) (compare with Table 35). For black, green, and peppermint teas the mean and maximum total PA concentrations reported in the study of Bodi et al. (2014) were somewhat lower than those reported in this study (Table 35), but this could be related to the fact that in this survey 28 PAs were monitored compared to only 17 in the study of Bodi et al. (2014).

Schulz et al. (2015) used LC-MS/MS to analyse 169 medicinal teas, that were commercially available on the German market, for the presence of 14 different PA FBs and 9 different PANOs. The study included 14 samples of chamomile and 4 of peppermint tea as well as a large number of mixed herbal teas (109). The reported LOQs (10 µg/kg for all PAs in dry tea) were significantly higher than in this

study (Table 16). This may be the reason why it was reported that only in around 50 % of the teas PAs were detected. The reported mean (253.4 µg/kg) and maximum (5 667.9 µg/kg) PA content in mixed herbal teas were comparable to the results obtained in this study (Table 35), but for the peppermint and chamomile teas much lower mean PA (8.9 and 4.6 µg/kg, respectively) and maximum PA (20.6 and 53.0 µg/kg, respectively) levels were found.

The study of Griffin et al. included a smaller number of (herbal) teas. Griffin et al. (2014) analysed 18 herbal dry teas available from the Irish market using LC-MS/MS. The method included 10 PA FBs and 4 PANOs and the LODs (0.4-1.5 µg/kg in dry tea) were comparable to this study (Table 16). Only in 50 % of the samples PAs were detected, but the mean and maximum contamination (210 and 1 733 µg/kg, respectively) were comparable to this study (Table 35), taking into account that only 14 PAs were monitored.

The study of Mathon et al. (2014) focused on the PA content of 70 (herbal) teas purchased from the Swiss market. The study included 10 black, 6 green, 10 chamomile, 8 peppermint, 9 rooibos and 15 mixed herbal teas that were analysed for 9 PA FBs by LC-MS/MS. Results were expressed as amount of PA/cup of infusion (200 ml), what makes a comparison with the current study somewhat less easy to make. LOQs reported were 0.02 µg/cup, which corresponds to approximately 10 µg/kg in dry tea. It was reported that 70 % of the tea infusions contained one or more PAs above the LOQ. No PAs were detected in black and green tea, but most chamomile and peppermint and all rooibos teas contained PAs. The reported mean PA content of the chamomile, peppermint and rooibos teas (190, 100 and 145 µg/kg, respectively) is substantially lower than in this study (Table 35), but this is likely due to the fact that PANOs were not analysed in the study of Mathon et al. (2014).

In conclusion, concerning the occurrence of individual PAs in the various types of tea the following can be summarised:

- Most of the PAs that were in the scope of the method were detected in the investigated tea samples, except for monocrotaline, its *N*-oxide and trichodesmine (except for a trace amount in a single tea). These PAs represent the monocrotaline-type with an eleven-membered macrocyclic ring (Figure 18).

- The most frequently occurring PAs were of the senecionine-type (senecionine-, retrorsine-, seneciphylline-, senecivernine-*N*-oxide and their respective free bases), found in 25 to 60 % of all samples. Erucifoline, jacobine and their respective *N*-oxides were detected in less than 5 % of samples (Figure 18).

- PAs of the lycopsamine and heliotrine-type were somewhat less frequently found (between 10 and 50 %): intermedine was the most common PA, followed by lycopsamine-*N*-oxide and heliotrine-*N*-oxide (Figure 18).

Concerning the mean concentrations of individual PAs in tea the following can be summarised:

- Senecionine-*N*-oxide was detected with the highest mean concentration (1.73 µg/L) and frequency (64 %) followed by retrorsine-, seneciphylline- and senecivernine-*N*-oxide and their respective free bases. These eight macrocyclic PAs together accounted for 76 % of total PA content determined in the investigated tea samples (Figure 18).

- The lycopsamine and heliotrine-type PAs together accounted for 24 % of the total PA content, while monocrotaline-type PAs were virtually absent.

- There was a strong co-occurrence of *N*-oxides and their respective free bases in the investigated teas. Furthermore, in most cases the *N*-oxide form of a certain PA was present in a higher concentration than the corresponding free base. This is in line with the general observation that PANOs predominate over PA free bases in PA-producing plants.

No significant differences in LODs were observed between the various PAs (see Table 15 in Section 8.2.1), and therefore, those PAs detected with the highest frequency and concentrations, seem to be the important ones with respect to PA contamination in tea.

14.2.2. Occurrence of PAs in food supplements

A summary of the results for food supplements is given in Table 36. Herbal supplements which were intended to be consumed as an infusion were analysed as ready-to-drink products (see Section 5.2.1) and these are reported as such in Table 37. To facilitate comparison with the other supplements the results for the herbal infusions are also expressed as in dry tea and as such are included in Table 36. For positive samples, the results for individual PAs in dry herbal supplements and supplements containing bee products are reported in Appendix I, and in herbal tea infusions in Appendix J.

Table 36: Total PA levels measured in different types of dry food supplements (sum of 28 individual PAs), in µg/kg

Type of food supplements	N	% of samples > LOD	Min. (µg/kg)	Max. (µg/kg)	Mean (µg/kg)	Median (µg/kg)
All supplements	191	60	<LOD	2 410 275	19 141	7.6
Bee products	29	66	<LOD	1 911.3	242.9	4.8
Supplements containing no PA- producing plants (dry products or plant extracts)	107	63	<LOD	8 488.1	317.6	11.4
Supplements containing PA-producing plants (dry products or plant extracts)	18	78	<LOD	2 410 275	196 534	39.0
Supplements containing no PA-producing plants (oil-based products)	3	0	<LOD	<LOD	<LOD	<LOD
Supplements containing PA-producing plants (oil-based products)	21	0	<LOD	<LOD	<LOD	<LOD
Supplements containing no PA-producing plants to be prepared as infusion [(a)]	1	100			62.2	
Supplements containing PA-producing plants to be prepared as infusion [(a)]	12	100	179.8	31 101	6 438.4	1 626.0

(a): Expressed as dry tea.

Table 37: Total PA levels measured in food supplements which were to be prepared as tea infusion (sum of 28 individual PAs), in µg/L

Type of food supplements	N	% of samples > LOD	Min. (µg/L)	Max. (µg/L)	Mean (µg/L)	Median (µg/L)
Tea infusion from supplement containing no PA-producing plants	1	100			0.83	
Tea infusion from supplement containing PA-producing plants	12	100	2.4	414.7	85.8	21.7

Supplements of bee products included pollen (n = 12), propolis (n = 9) and royal jelly (n = 8) samples. In eleven of the twelve pollen products PAs were detected and the mean concentration was 576.0 µg/kg, while 0.6 and 15.5 µg/kg were quantified in propolis and royal jelly products. In Figure 24 the distribution of PAs in bee products is shown. Bee products mainly contained PAs of the lycopsamine-type, including echimidine, intermedine, lycopsamine, together with smaller amounts of senecionine-type PAs. The highest PA mean concentrations were determined for echimidine and echimidine-*N*-oxide. These results and PA patterns are comparable to those known from honey beside the fact that the respective *N*-oxide form is generally not detectable in (blended) honey. Although only a limited number of bee products of organic production were investigated no differences in the PA content were observed for supplements from organic or non-organic production.

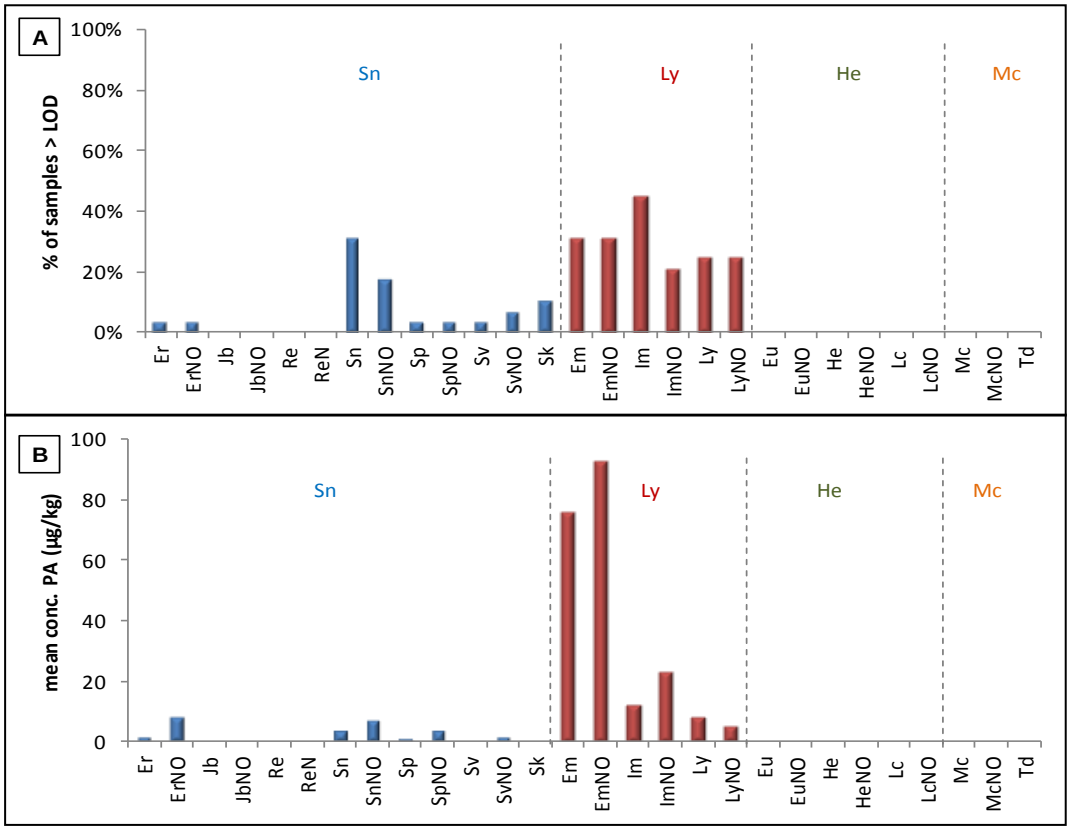

Figure 24: (A) Percentage of 29 bee product samples containing an individual PA > LOD and (B) mean concentrations of individual PAs in the investigated bee product samples. Both graphs are separated into four categories – summarising senecionine-type (Sn), lycopsamine-type (Ly), heliotrine-type (He) and monocrotaline-type (Mc) PAs, respectively.

A total of 107 herbal food supplements was investigated, samples that according to the label should not contain any ingredients of known PA-producing plants. Some of these supplements contained material of a single plant species, but others could be mixtures of up to 10 or more different plant species. Nevertheless, in 63 % of the samples PAs were detected with a mean concentration of 317.6 and a median value of 11.4 µg/kg. Such high differences between mean and median concentrations indicate that only a few samples contain comparatively high PA concentrations while most of the samples exhibit considerably lower PA contents. All four PA types: the senecionine-type (Sn), lycopsamine-type (Ly), heliotrine-type (He) and monocrotaline-type (Mc) were detected, while the highest PA concentrations were determined for the lycopsamine type (Figure 25). Lycopsamine-type

PAs are synthesised by all genera of the *Boraginaceae* family as well as by the genus of *Eupatorium* of the *Asteraceae* family.

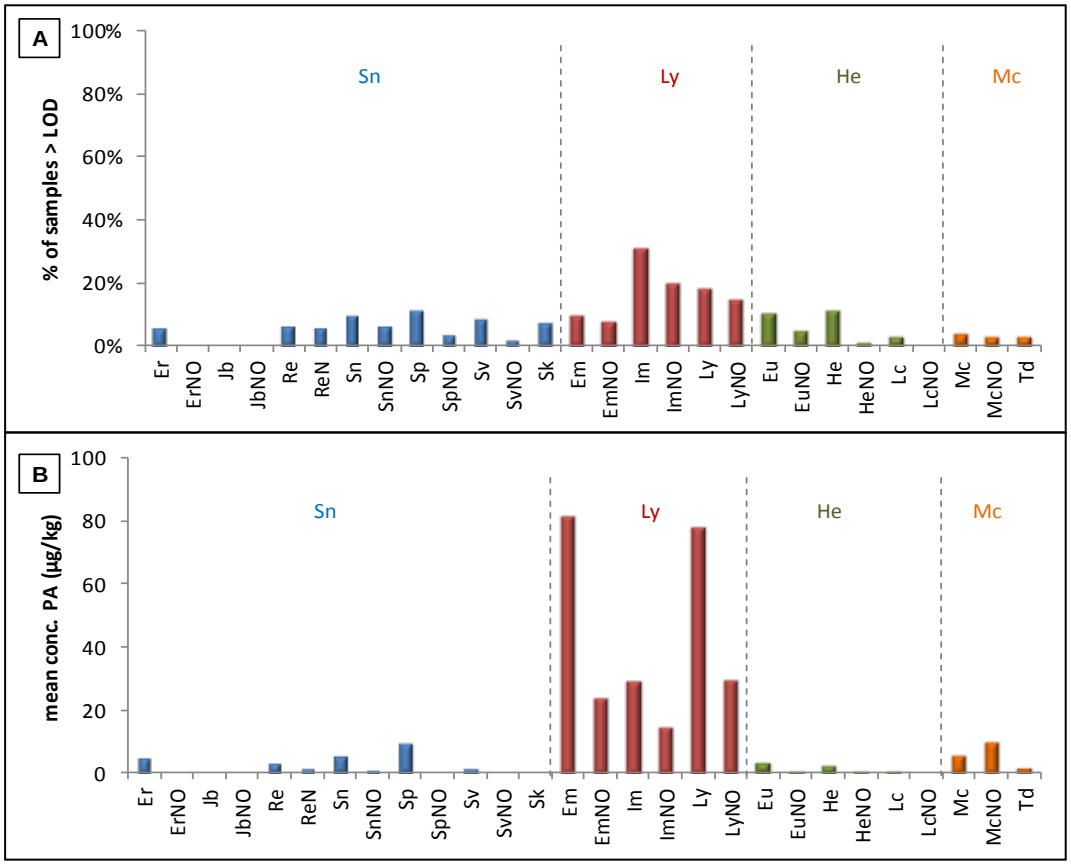

Figure 25: (A) Percentage of 107 samples of non PA-producing plants containing an individual PA > LOD and (B) mean concentrations of individual PAs in the investigated samples of non PA-producing plant. Both graphs are separated into four categories – summarising senecionine-type (Sn), lycopsamine-type (Ly), heliotrine-type (He) and monocrotaline-type (Mc) PAs, respectively.

For two groups of food supplements a more detailed analysis could be made, due to the fact that a somewhat larger set of samples was collected.

There were 18 food supplements that contained Valerian (*Valeriana officinale*) root powder or extract as a single or as an important component. Interestingly, only in 5 out of the 18 Valerian products PAs could be detected, 4 of them only containing trace amounts of PAs (< 50 µg/kg). The mean concentration of PAs was 37.1 µg/kg and only PA FBs were detected, belonging to different PA-types (data not shown).

The second individual product group of supplements of interest were those containing St. John's wort (*Hypericum perforatum*) products, of which 14 samples were collected. In all samples but one, PAs were detected with a mean concentration of 991.7 µg/kg and a median concentration of 734.8 µg/kg. The PA profile is dominated by lycopsamine-type PAs. Echimidine and its *N*-oxide are present in the highest concentration (Figure 26). St. John's wort products appear thus often contaminated with (traces of) plant material from species of the *Boraginaceae* family or the *Eupatorium* genus.

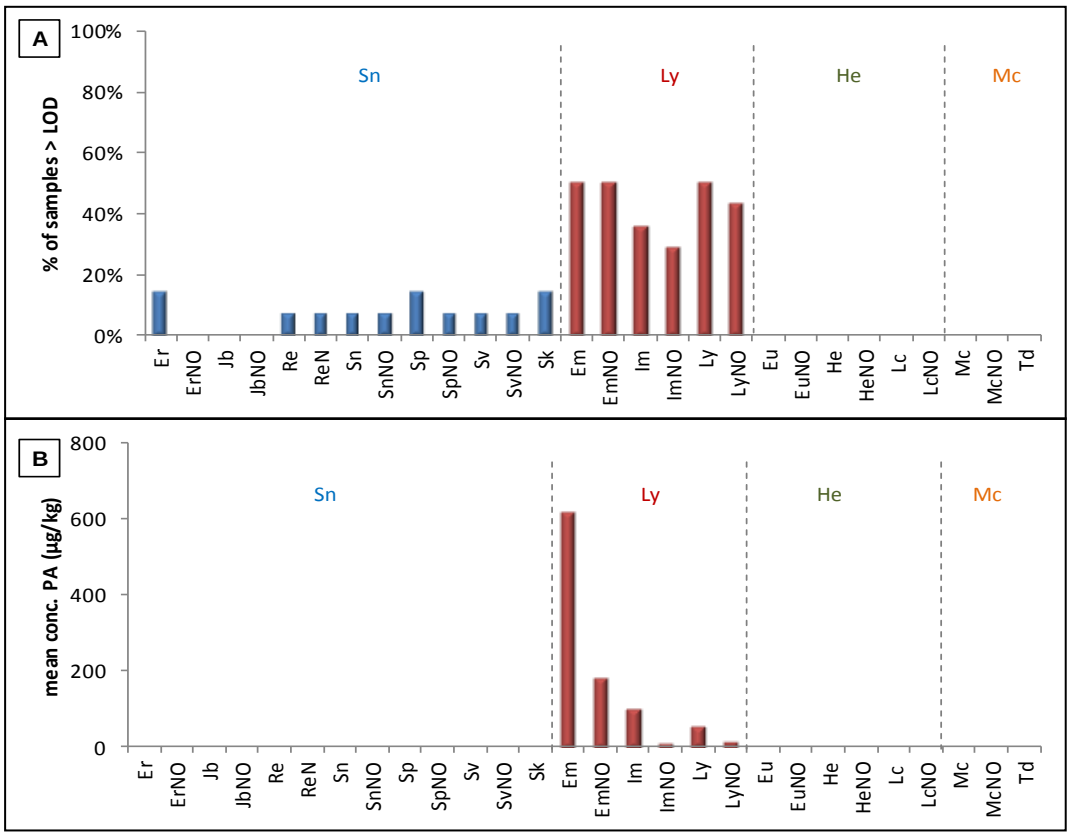

Figure 26: (A) Percentage of 14 St. John's wort samples containing an individual PA > LOD and (B) mean concentrations of individual PAs in the investigated St. John's wort samples. Both graphs are separated into four categories – summarising senecionine-type (Sn), lycopsamine-type (Ly), heliotrine-type (He) and monocrotaline-type (Mc) PAs, respectively.

In traditional medicine dried parts and extracts of plants and herbs are often used as homeopathic preparations. Applications of PA-producing plants are also in use as for instance tea infusion of leaves of coltsfoot (*Tussilago farfara*) as cough-relieving mixture or comfrey (*Symphytum officinale*) as anti-inflammatory and decongestant drug. Also essential oil extracts of borage (*Borago officinalis*) and blueweed (*Echium vulgare* or *E. plantagineum*) seed are available. All these products are sold as food supplements and 51 samples were investigated. Of these samples 30 consisted of dry plant material or liquid extracts, while 21 of them were oil-based extracts.

Most of the oil-based supplements were produced from *Borago* (n = 19) and two from *Echium* species. Due to the hydrophilic structure of PAs it is expected that PAs are only co-extracted to a minor content in the lipophilic oil fraction of the plants or seeds. In accordance with this expectation, no PAs were quantified in any these oils from PA plants (Table 36).

Supplements based on dry material from PA-producing plants were subdivided into directly ingested drugs (n = 18) and supplements intended to be prepared as tea infusion (n = 12). The supplements for direct use contained PA concentrations (sum of) ranging from < LOD up to 2 410 275 µg/kg (see Tables 36 and 38), while for the supplements to be consumed as tea infusion concentrations ranged from 2.4 to 414.7 µg/L (see Table 37). Expressed as dry tea the latter group ranged from 179.8 to 31 101 µg/kg (Tables 36 and 38).

Table 38: Total PA levels measured in 30 food supplements containing plant material based on PA-producing plants (sum of 28 individual PAs), in µg/kg. For food supplements that are to be consumed as infusion the concentrations are expressed as on dry plant material basis

Sample code	Product	Organic Y/N	Sum PAs (µg/kg)	Genus	Plant Family [(a)]	PA-Type
FP14/0887	Borraja Planta (*Borrago officinalis*)*	Yes	31 101	*Borago*	Borag.	Ly
FP14/0890	Borrajas (*Borrago officinalis*)*	No	28 692	*Borago*	Borag.	Ly
FP14/0809	Eupatoire Plante (*Eupatorium can.*)	No	2 410 275	*Eupatorium*	Aster.	Ly
FP14/0748	Boneset powder (*Eupatorium per.*)	No	1 077 547	*Eupatorium*	Aster.	Ly
FP14/0792	*Eupatorium odoratum* Linn. Herbal tea*	No	545.0	*Eupatorium*	Aster.	Ly
FP14/0787	Tablets containing *Eupatorium perfolatum*	No	50.8	*Eupatorium*	Aster.	Ly
FP14/0788	Tablets containing *Eupatorium per.*	No	15.8	*Eupatorium*	Aster.	Ly
FP14/0808	Grémil Plante (*Lithospermum officinale*)	No	14 557	*Lithospermum*	Borag.	Ly
FP14/0889	Mill del Sol (*Lithospermum officinalis*)*	No	4163.6	*Lithospermum*	Borag.	Ly
FP14/0884	Common Gromwell (*Litosphermum off.*)	No	1 022.9	*Lithospermum*	Borag.	Ly
FP14/0793	Longkruid (*Pulmonaria officinalis*)*	Yes	1885.3	*Pulmonaria*	Borag.	Ly
FP14/0832	Pulmonaria (*Pulmonaria officinalis*)*	Yes	1720.0	*Pulmonaria*	Borag.	Ly
FP14/0885	Pulmonaria (*Pulmonaria officinalis*)*	No	803.9	*Pulmonaria*	Borag.	Ly
FP14/0805	Lungwort (*Pulmonaria officinalis*)*	No	675.0	*Pulmonaria*	Borag.	Ly
FP14/0891	Lungwort (*Pulmonaria officinalis*) and other herbs*	No	372.6	*Pulmonaria*	Borag.	Ly
FP14/0750	Lungwort powder (*Pulmonaria officinalis*)	No	256.9	*Pulmonaria*	Borag.	Ly
FP14/0886	Lungwort (*Pulmonaria officinalis*)*	Yes	179.8	*Pulmonaria*	Borag.	Ly
FP14/0749	Comfrey (*Symphytum off.*) leaves powder	No	17 610	*Symphytum*	Borag.	Ly
FP14/0834	Consuelda (Comfrey) *(Symphytum off.*)	No	0.0	*Symphytum*	Borag.	Ly
FP14/0730	Tincture - comfrey leaves (*Symphytum off.*)	Yes	0.0	*Symphytum*	Borag.	Ly
FP14/0728	Butterbur (*Petasitis sp.*) - neurological support	No	27.1	*Petasites*	Aster.	Sn
FP14/0762	Butterbur extra (*Petasites sp.*)	No	6.2	*Petasites*	Aster.	Sn
FP14/0881	Purple butterbur (*Petasitis hybridus*) root extract	No	2.8	*Petasites*	Aster.	Sn
FP14/0801	Butterbur (*Petasitis hybridicus*) standardized root extract	No	0.0	*Petasites*	Aster.	Sn
FP14/0897	Purple butterbur (*Petasitis hybridus*) root extract	No	0.0	*Petasites*	Aster.	Sn
FP14/0898	Coltsfoot (*Tussilage farfara*) leaf extract	No	15 769	*Tussilago*	Aster.	Sn
FP14/0806	Klein Hoefblad (*Tussilago Farfarae*)*	No	5590.8	*Tussilago*	Aster.	Sn
FP14/0879	Coltsfoot (*Tussilago farfarae*)*	No	1532.1	*Tussilago*	Aster.	Sn
FP14/0831	Huflattich (*Tussilago Farfarae*)	Yes	471.6	*Tussilago*	Aster.	Sn
FP14/0851	Sirup containing coltsfoot (*Tussilago far.*)	No	4.2	*Tussilago*	Aster.	Sn

* Supplements which were intended to be consumed as an infusion (analysed as ready-to-drink products). PA concentrations have been expressed as dry supplement.
(a): Aster. = Asteraceae, Borag. = Boraginaceae.

Two herbal supplements containing *Eupatorium sp.* plant material (FP14/0809 and FP14/0748) contained PAs in a proportion of the dry mass of 0.24 and 0.11 %, respectively. These values are well

within the range of PA concentrations of producing plants reported in literature (Molyneux et al., 1979; Stegelmeier, 2011; These et al., 2013).

As shown in Figure 27, the investigated food supplements of PA-producing plants mainly contain PAs of the lycopsamine-type, except plants of the genus *Petasites* and *Tussilago* from the *Asteraceae* family (Table 38), which produce senecionine-type PAs, although the most frequently formed PA is senkirkine, which is an otonecine-type PA with regard to the necine base. This is reflected in the results as predominantly the monoesters lycopsamine, intermedine and their respective *N*-oxides as well as the cyclic diester senkirkine could be detected (Figure 27).

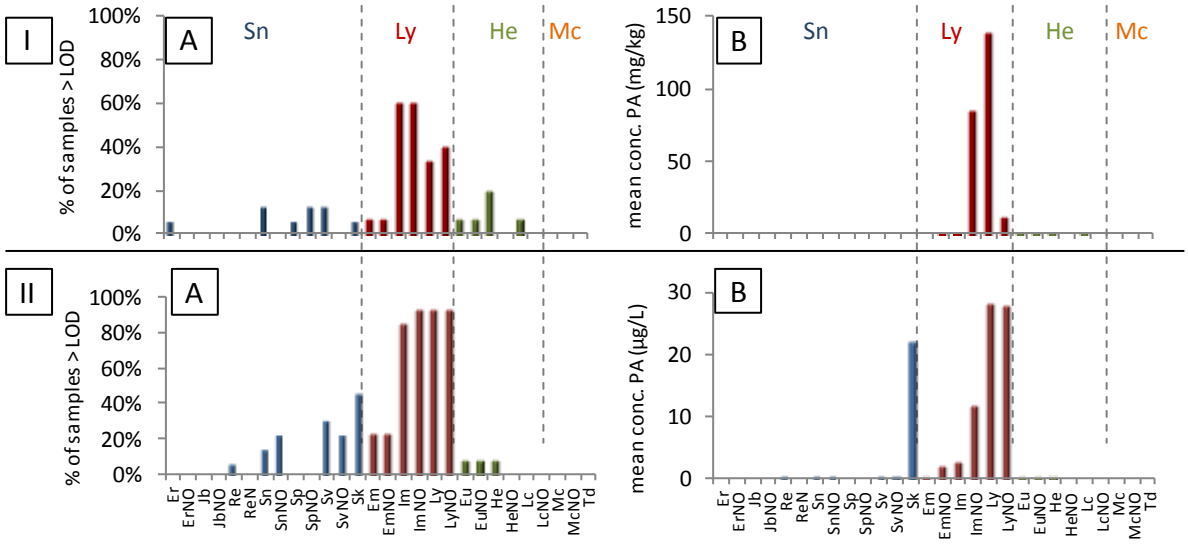

Figure 27: (A) Percentage of PA-producing plant samples containing an individual PA > LOD and (B) mean concentrations of individual PAs in investigated PA-producing plant samples either intended for direct use as drug (I) or intended to be ingested as infusion (II). Both graphs are separated into four categories – summarising senecionine-type (Sn), lycopsamine-type (Ly), heliotrine-type (He) and monocrotaline-type (Mc) PAs, respectively.

In conclusion, concerning the occurrence of individual PAs in food supplements the following can be summarised:

- Many of the investigated food supplement samples (60 %) contained PAs. Predominantly PAs of the lycopsamine-type and lesser amounts of the senecionine-type were detected.
- Supplements containing oil-based extracts from PA-producing as well as from non PA-producing plants, were free of PAs.
- With regard to the individual detected PAs, depending on the plant species used in the PA-plant containing supplements, lycopsamine, intermedine and their respective *N*-oxides, as well as senkirkine, were found in the highest concentrations (Figure 27).
- Supplements that did not contain PA-producing plants as natural ingredients revealed a comparable level of PA contamination as (herbal) teas. The PA pattern was however more limited, with a predominance of lycopsamine-type PAs (Figure 25).

- Relatively high PA concentrations could be detected in supplements containing St. John's wort, where all samples but one, contained PAs of the lycopsamine type (Figure 26).

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- The aim of this study to assess the occurrence of pyrrolizidine alkaloids (PAs) in animal-derived products such as milk, eggs and meat for human consumption, as well as in plant-derived products such as (herbal) teas and (herbal) food supplements, across different regions in Europe, has been successfully achieved.

- Two analytical methods based on multi-analyte LC-MS/MS have been satisfactory validated to detect and accurately quantify 35 different PAs in the animal-derived samples and 28 different PAs in plant-derived samples at the low performance levels that are required.

- Quality control data showed adequate performance of the analytical methods for milk and eggs, and sufficient stability of the milk and egg QC samples. The performance of the analytical method with respect to meat samples was not for all matrices fully satisfactory, as variable recoveries and matrix suppression/interferences were encountered, in particular for bovine meat.

- A total of 1 105 samples have been collected and analysed for the presence of PAs. This included 746 samples of animal origin (268 samples of milk and milk products, 205 egg samples and 273 samples of meat and meat products) and 359 samples of plant origin (168 samples of (herbal) teas and 191 herbal food supplements).

- Analysis of the animal-derived products revealed occasional low levels of PAs in milk samples (6 %), mostly with single PAs (i.e. jacoline, senkirkine, otosenine, lycopsamine, echimidine, retrorsine) in their free base form. Except for two egg samples, PAs were absent in the milk products, eggs, meat and liver samples analysed.

- The analysis of the (herbal) tea samples revealed that a high proportion of (herbal) teas (91 %) contained one or more PAs. The mean concentration for the sum of 28 PAs was 6.13 µg/L tea, with a maximum of 64.0 µg/L. Of the various types of tea, rooibos tea showed the highest concentration (mean PA concentration of 7.99 µg/L), while chamomile tea on average contained the lowest PA concentration (3.67 µg/L). PAs belonging to the senecionine-type (senecionine, retrorsine, seneciphylline) were the most frequently found. The *N*-oxide forms generally were present in higher concentrations than the free base forms.

- Food supplements were often contaminated with PAs (60 %), but the concentrations were highly variable. As expected, the highest PA levels were found in herbal food supplements made from plant material of known PA producers. Supplements containing oil-based extracts of PA-producing plants were generally free of PAs. In the food supplements, PAs belonging to the lycopsamine-type (lypcosamine, intermedine, echimidine) were the most frequently found. PAs were often present as mixtures of free bases and *N*-oxides.

RECOMMENDATIONS

- More information is needed on the occurrence of PAs in (herbal) teas and (herbal) food supplements. In particular, the source and route of contamination are still largely unknown

and need further investigation to reduce the contamination levels of tea and herbal supplements and to reduce the exposure of consumers.

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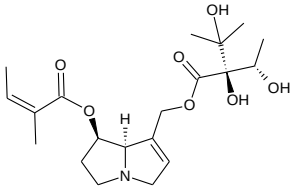
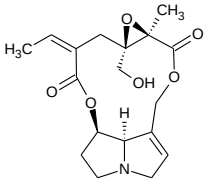
APPENDICES

Appendix A. Chemical structures of pyrrolizidine alkaloids (PAs)

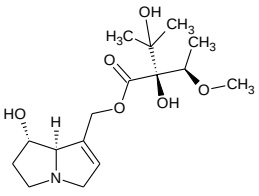
A.1. PA Free bases (PA FBs)



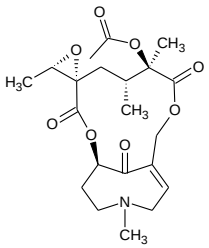
Echimidine
$C_{20}H_{31}NO_7$
Mass: 397.210 Da
[M+H]+: 398.217 Da



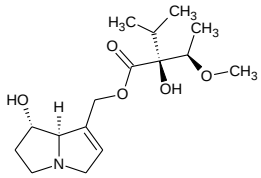
Erucifoline
$C_{18}H_{23}NO_6$
Mass: 349.153 Da
[M+H]+: 350.160 Da



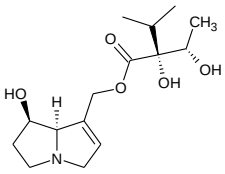
Europine
$C_{16}H_{27}NO_6$
Mass: 329.184 Da
[M+H]+: 330.191 Da



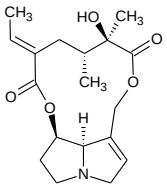
Florosenine
$C_{21}H_{29}NO_8$
Mass: 423.189 Da
[M+H]+: 424.197 Da



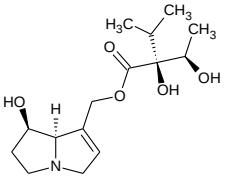
Heliotrine
$C_{16}H_{27}NO_5$
Mass: 313.189 Da
[M+H]+: 314.196 Da



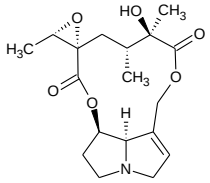
Indicine
$C_{15}H_{25}NO_5$
Mass: 299.173 Da
[M+H]+: 300.181 Da



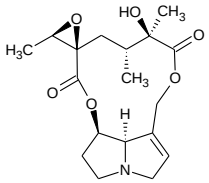
Integerrimine
$C_{18}H_{25}NO_5$
Mass: 335.173 Da
[M+H]+: 336.181 Da



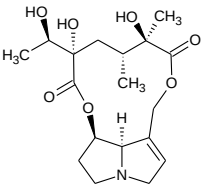
Intermedine
$C_{15}H_{25}NO_5$
Mass: 299.173 Da
[M+H]+: 300.181 Da



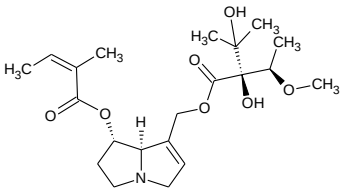
Jacobine
$C_{18}H_{25}NO_6$
Mass: 351.168 Da
[M+H]+: 352.175



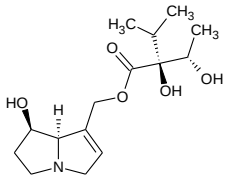
epi-Jacobine (IS)
$C_{18}H_{25}NO_6$
Mass: 351.168 Da
[M+H]+: 352.175



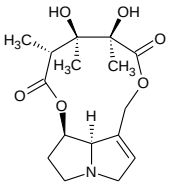
Jacoline
$C_{18}H_{27}NO_7$
Mass: 369.179 Da
[M+H]+: 370.186 Da



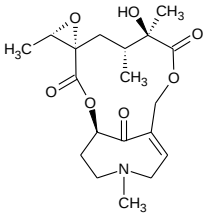
Lasiocarpine
$C_{21}H_{33}NO_7$
Mass: 411.226 Da
[M+H]+: 412.233 Da



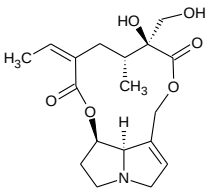
Lycopsamine
$C_{15}H_{25}NO_5$
Mass: 299.173 Da
[M+H]+: 300.181 Da



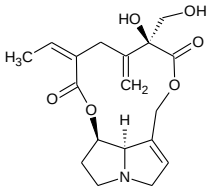
Monocrotaline
$C_{16}H_{23}NO_6$
Mass: 325.153 Da
[M+H]+: 326.160 Da



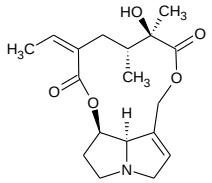
Otosenine
$C_{18}H_{27}NO_7$
Mass: 381.179 Da
[M+H]+: 382.186 Da



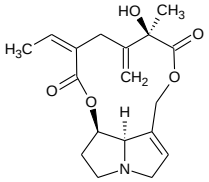
Retrorsine
$C_{18}H_{25}NO_6$
Mass: 351.168 Da
[M+H]+: 352.175 Da



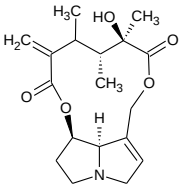
Riddelliine
$C_{18}H_{23}NO_6$
Mass: 349.153 Da
[M+H]+: 350.160 Da



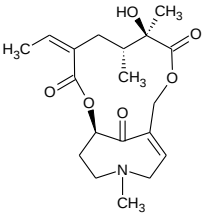
Senecionine
$C_{18}H_{25}NO_5$
Mass: 335.173 Da
[M+H]+: 336.181 Da



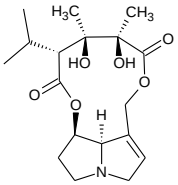
Seneciphylline
$C_{18}H_{23}NO_5$
Mass: 333.158 Da
[M+H]+: 334.165 Da



Senecivernine
$C_{18}H_{25}NO_5$
Mass: 335.173 Da
[M+H]+: 336.181 Da

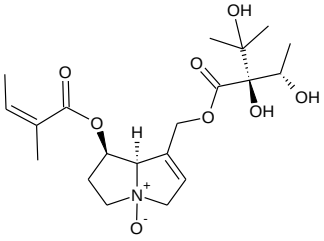


Senkirkine
$C_{19}H_{27}NO_6$
Mass: 365.184 Da
[M+H]+: 366.191 Da

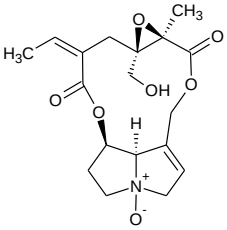


Trichodesmine
$C_{18}H_{27}NO_6$
Mass: 353.184 Da
[M+H]+: 354.191 Da

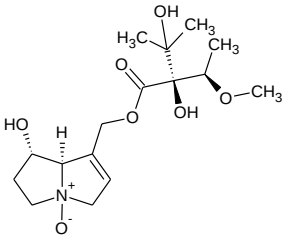
A.2. PA *N*-oxides (PANOs)



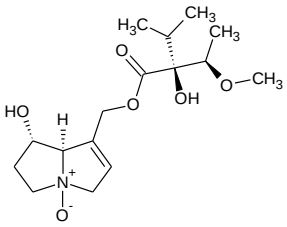
Echimidine-*N*-oxide
$C_{20}H_{31}NO_8$
Mass: 413.205 Da
[M+H]+: 414.212 Da



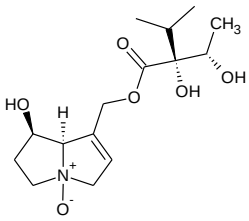
Erucifoline-*N*-oxide
$C_{18}H_{23}NO_7$
Mass: 365.147 Da
[M+H]+: 366.155 Da



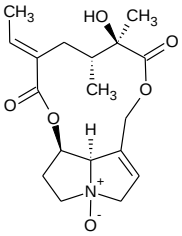
Europine-*N*-oxide
$C_{16}H_{27}NO_7$
Mass: 345.179 Da
[M+H]+: 346.186 Da



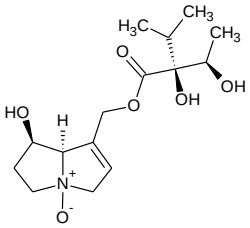
Heliotrine-*N*-oxide
$C_{16}H_{27}NO_6$
Mass: 329.184 Da
[M+H]+: 330.191 Da



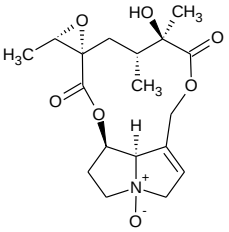
Indicine-*N*-oxide
$C_{15}H_{25}NO_6$
Mass: 315.168 Da
[M+H]+: 316.175 Da



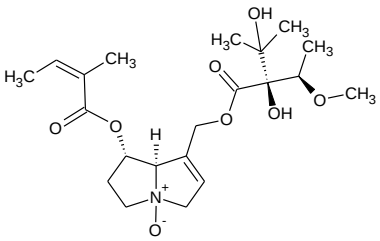
Integerrimine-*N*-oxide
$C_{18}H_{25}NO_6$
Mass: 351.168 Da
[M+H]+: 352.175 Da



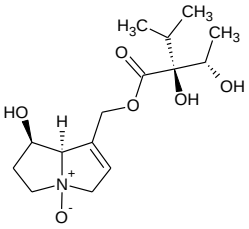
Intermedine-*N*-oxide
$C_{15}H_{25}NO_6$
Mass: 315.168 Da
[M+H]+: 316.175 Da



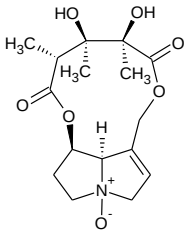
Jacobine-*N*-oxide
$C_{18}H_{25}NO_7$
Mass: 367.163 Da
[M+H]+: 368.170



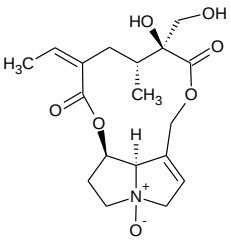
Lasiocarpine-*N*-oxide
$C_{21}H_{33}NO_8$
Mass: 427.221 Da
[M+H]+: 428.228 Da



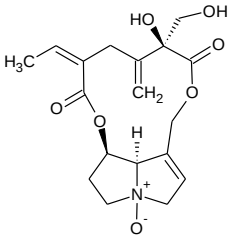
Lycopsamine-*N*-oxide
$C_{15}H_{25}NO_6$
Mass: 315.168 Da
[M+H]+: 316.175 Da



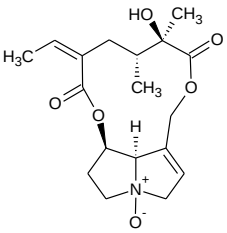
Monocrotaline-*N*-oxide
$C_{16}H_{23}NO_7$
Mass: 341.147 Da
[M+H]+: 342.155 Da



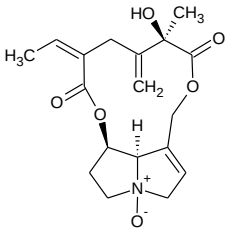
Retrorsine-*N*-oxide
$C_{18}H_{25}NO_7$
Mass: 367.163 Da
[M+H]+: 368.170 Da



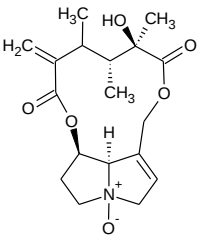
Riddelliine-*N*-oxide
$C_{18}H_{23}NO_7$
Mass: 365.147 Da
[M+H]+: 366.155 Da



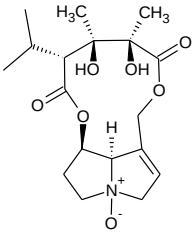
Senecionine-*N*-oxide
$C_{18}H_{25}NO_6$
Mass: 351.168 Da
[M+H]+: 352.175 Da



Seneciphylline-*N*-oxide
$C_{18}H_{23}NO_6$
Mass: 349.153 Da
[M+H]+: 350.160 Da



Senecivernine-*N*-oxide
$C_{18}H_{25}NO_6$
Mass: 351.168 Da
[M+H]+: 352.175 Da



Trichodesmine-*N*-oxide
$C_{18}H_{27}NO_7$
Mass: 369.179 Da
[M+H]+: 370.186 Da

Appendix B. Linearity of the LC-MS/MS analysis of PAs in (herbal) teas

The verification of the linearity within the concentration range used for analysis based on the goodness-of-fit-test according to Mandel (DIN, 1986).

Calculation of the test value PW

$$PW = \frac{(n-2)*S_{y1}{}^2 - (n-3)*S_{y2}{}^2}{S_{y2}}$$

DS^2 squared differences of variances
S_{y1} standard deviation of calibration function of first order
Sy_2 standard deviation of calibration function of second order
n number of measurement values

F-value = 8.86 with n = 17 (all PA except retrorsine-*N*-oxide) and F = 8.68 with n = 15 (retrorsine-*N*-oxide)

The calibration data obtained in herbal tea are shown in Table B.1. For each analyte (except retrorsine-*N*-oxide) a test value below the required reference value was obtained. As R^2 of the calibration curve for retrorsine-*N*-oxide is still above 0.99 a linear regression can still be assumed. Therefore, the calibration curves for all analytes were considered to be linear over a concentration range of 1-300 µg/kg.

Table B.1. Results of Mandel test and coefficient of determination (R^2) of 9-point calibration curve in herbal tea (LOD to 150 ng/mL representing a PA concentration of LOD-300 µg/kg dry product)

Analyte	Test value PW (reference F-value is 8.86/8.68)	coefficient of determination (R^2)
Em	2.88	0.9978
He	0.96	0.9966
HeNO	6.32	0.9986
Im	1.22	0.9958
Ly	7.58	0.9975
Lc	2.18	0.9989
LcNO	3.12	0.9985
Mc	3.18	0.9976
McNO	3.52	0.9947
Re	6.02	0.9976
ReNO	6.86	0.9982
Sn	0.18	0.9984
SnNO	0.57	0.9990
Sk	0.00	0.9986
Sp	1.33	0.9981
SpNO	1.17	0.9963
Td	0.16	0.9964

Appendix C. Comparison of PA standards between RIKILT and BfR

Table C.1. Purity of the individual PA standards (assessed by BfR)

Pyrrolizidine alkaloid	Abbrev.	Contamination	
Echimidine	Em	EmNO (0.23 %)	
Echimidine-*N*-oxide	EmNO	Em (3.63 %)	
Erucifoline	Er	n.d.	
Erucifoline-*N*-oxide	ErNO	n.d.	
Europine	Eu	He (8.41 %)	Lc (0.54 %)
Europine-*N*-oxide	EuNO	Eu (0.07 %)	
Heliotrine	He	HeNO (0.51 %)	
Heliotrine-*N*-oxide	HeNO	He (0.12 %)	
Intermedine	Im	Ly (1.89 %)	
Intermedine-*N*-oxide	ImNO	Im (0.60 %)	
Jacobine	Jb	n.d.	
Jacobine-*N*-oxide	JbNO	n.d.	
Lasiocarpine	Lc	LcNO (0.25 %)	
Lasiocarpine-*N*-oxide	LcNO	n.d.	
Lycopsamine	Ly	n.d.	
Lycopsamine-*N*-oxide	LyNO	Ly (1.73 %)	
Monocrotaline	Mc	McNO (0.51 %)	
Monocrotaline-*N*-oxide	McNO	n.d.	
Retrorsine	Re	ReNO (0.47 %)	
Retrorsine-*N*-oxide	ReNO	n.d.	
Senecionine	Sn	n.d.	
Senecionine-*N*-oxide	SnNO	n.d.	
Seneciphylline	Sp	n.d.	
Seneciphylline-*N*-oxide	SpNO	n.d.	
Senecivernine	Sv	n.d.	
Senecivernine-*N*-oxide	SvNO	Sv (0.16 %)	
Senkirkine	Sk	n.d.	
Trichodesmine	Td	n.d.	

n.d. = not detected.

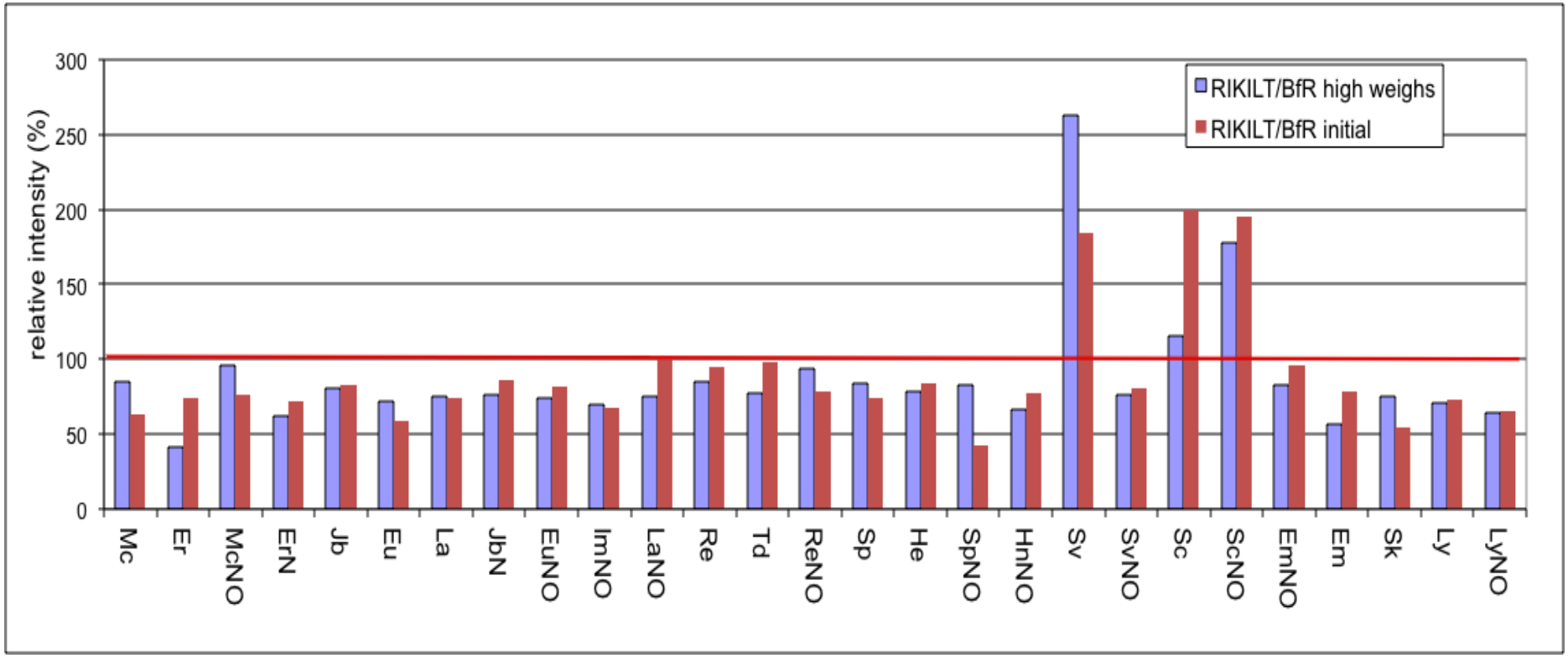


Figure C.1. Comparison between RIKILT and BfR standards. Red bars were the differences (%) found in the initial experiment and blue bars are the differences found when higher amount of standards were weighed to prepare the individual stock solutions. Abbreviations are explained in Table 10.

Appendix D. Results for the inter-laboratory study between RIKILT and IRTA

Table D.1. Results of the inter-laboratory study between RIKILT and IRTA for milk samples. Abbreviations are explained in Table 10.

a) PA FBs

PA FB:		Em	Er	Eu	Fs	He	Ir	Jb	Jl	Lc	Ly	Mc	Ot	Re	Rd	Sn	Sp	Sv	Sk	Td	Avg	SD
Recovery (%) (n = 3)	IRTA	99.9	96.7	91.0	103.2	104.7	95.7	87.3	87.7	92.7	88.1	86.7	100.1	101.3	80.9	91.4	98.0	90.7	99.9	97.3	94.4	2.4
	RIKILT	95.5	88.0	91.0	98.6	87.9	92.1	89.2	80.0	93.9	81.5	79.6	100.0	88.4	86.1	85.2	92.8	90.4	98.6	87.8	89.8	4.6
RSD (%) (n = 3)	IRTA	2.2	3.3	1.8	2.6	1.6	1.6	2.0	0.6	1.7	1.3	0.9	1.1	3.4	13.2	2.0	3.2	1.3	1.8	1.7		
	RIKILT	7.6	3.1	5.7	2.8	5.9	7.6	5.2	2.1	4.8	2.5	1.5	0.3	3.4	6.0	12	5.2	12.2	2.6	6.9		
Accuracy (%) 2.5 µg/L	IRTA	93.9	98.3	104.4	94.2	91.6	95.5	113.6	89.2	95.7	92.3	97	95.2	92.0	163.3	96.0	88.3	100.2	95.5	91.9	99.4	16.5
	RIKILT	103.6	97.9	99.1	98.7	101.9	101.3	106.6	105.8	100.6	102.9	99.8	100.6	105.2	100.8	116.2	98.8	108.9	97.3	99.6	102.4	4.6

b) PANOs

PANO:		EmNO	ErNO	EuNO	HeNO	IdNO	IrNO	JbNO	LcNO	LyNO	McNO	ReNO	RdNO	SnNO	SpNO	SvNO	TdNO	Avg	SD
Recovery (%) (n = 3)	IRTA	105.8	88.9	98.1	96.2	92.3	97.2	99.8	106.3	91.6	103.9	97.2	97.3	102.1	91.6	101.5	95.9	97.9	2.4
	RIKILT	101.0	96.2	90.9	101.5	81.4	102.3	96.8	97.9	87.7	75.6	99.8	78.5	94.7	93.2	101.1	99.6	93.6	2.5
RSD (%) (n = 3)	IRTA	3.8	4.7	2.2	1.3	4.9	0.6	3.5	2.5	1.3	1.1	3.0	0.7	1.5	0.9	4.8	1.8		
	RIKILT	1.4	3.6	2.4	3.6	2.5	1.7	1.5	1.6	9.0	3.1	4.3	1.7	1.7	1.5	2.5	1.8		
Accuracy (%) 2.5 µg/L	IRTA	96.3	94.7	102.7	93.4	104.0	94.9	104.4	91.0	102.1	93.7	96.5	100.3	96.0	96.7	96.3	99.9	97.7	4.0
	RIKILT	102.5	99.0	100.1	99.9	100.1	98.3	99.4	94.5	97.1	101.7	99.7	101.9	97.9	100.3	96.8	98.7	99.2	2.1

Table D.2. Results of the inter-laboratory study between RIKILT and IRTA for egg samples. Abbreviations are explained in Table 10.

a) PA FBs

PA FB:		Em	Er	Eu	Fs	He	Ir	Jb	Jl	Lc	Ly	Mc	Ot	Re	Rd	Sn	Sp	Sv	Sk	Td	Avg	SD
Recovery (%)	IRTA	79.6	86.4	85.8	86.0	84.9	74.7	83.1	87.1	70.6	85.5	88.2	88.2	83.5	81.8	76.7	77.1	74.6	89.9	83.0	82.5	2.1
(n = 3)	RIKILT	80.1	73.3	80.4	87.6	82.7	77.3	76.0	48.4	82.7	61.6	48.8	89.4	77.6	74.2	74.2	73.7	72.8	91.3	78.4	75.3	2.4
RSD (%)	IRTA	1.0	3.4	0.6	3.1	2.7	2.3	4.7	1.1	1.0	1.8	2.2	1.9	2.6	3.5	2.5	6.3	1.4	1.7	3.4		
(n = 3)	RIKILT	3.0	2.1	3.8	0.8	3.6	3.2	1.5	4.5	3.8	1.0	0.9	3.0	2.4	3.7	3.4	3.5	3.8	5.5	3.6		
Accuracy (%)	IRTA	94.4	101.7	103.1	96.4	102.9	99.6	99.7	100.7	96.7	96.8	100.2	101.9	94.8	94.1	97.4	94.9	98.9	97.2	99.7	98.5	2.9
10 µg/kg	RIKILT	102.4	81.7	75.9	103.0	88.7	94.7	82.7	63.7	108.2	72.7	62.9	95.2	85.2	86.5	97.6	92.3	97.4	99.5	4.6	88.6	12.8

b) PANOs

PANO:		EmNO	ErNO	EuNO	HeNO	IdNO	IrNO	JbNO	LcNO	LyNO	McNO	ReNO	RdNO	SnNO	SpNO	SvNO	TdNO	Avg	SD
Recovery (%)	IRTA	85.9	86.6	92.3	91.0	88.2	90.9	90.3	83.4	91.8	90.3	89.3	84.7	91.5	86.9	90.2	91.0	89.0	2.0
(n = 3)	RIKILT	91.2	75.8	70.5	80.0	63.3	93.8	79.9	90.0	62.4	42.7	90.2.	53.2	95.9	73.6	94.9	96.3	78.4	3.2
RSD (%)	IRTA	1.8	1.4	2.7	0.1	2.4	0.8	2.6	3.9	1.1	3.0	3.7	1.0	4.6	0.6	3.3	3.5		
(n = 3)	RIKILT	2.4	4.2	1.4	0.8	1.3	2.7	5.1	1.3	1.9	3.4	5.1	7.0	3.1	1.2	6.1	15.5		
Accuracy (%)	IRTA	100.6	100.6	102.0	97.2	104.4	103.1	105.2	100.9	101.3	99.7	100.3	99.7	99.8	103.0	100.1	101.9	101.2	2.0
10 µg/kg	RIKILT	98.3	93.2	87.6	97.7	87.1	97.8	97.4	104.6	88.9	88.1	94.5	104.3	101.3	97.4	101.1	93.4	95.8	5.7

Table D.3. Results of the inter-laboratory study between RIKILT and IRTA for poultry meat samples. Abbreviations are explained in Table 10.

a) PA FBs

PA FB:		Em	Er	Eu	Fs	He	Ir	Jb	Jl	Lc	Ly	Mc	Ot	Re	Rd	Sn	Sp	Sv	Sk	Td	Avg	SD
Recovery (%)	IRTA	47.9	51.0	48.1	50.7	52.0	51.6	51.0	40.6	46.9	44.4	44.5	50.6	52.5	49.7	50.9	52.1	49.0	50.3	53.6	49.3	2.4
(n = 3)	RIKILT	57.8	75.4	68.2	62.5	61.2	57.4	61.4	59.1	55.6	64.6	63.5	69.3	63.3	59.3	57.2	64.9	54.3	78.9	60.6	62.9	6.4
RSD (%)	IRTA	1.4	7.7	7.6	3.0	6.4	1.7	7.3	8.8	3.0	4.1	8.5	3.6	4.6	3.2	0.1	0.9	0.4	2.7	2.2		
(n = 3)	RIKILT	1.7	4.3	1.4	2.8	3.4	1.6	6.2	1.4	3.1	2.4	2.7	2.1	3.1	3.3	1.1	2.1	2.3	5.1	2.2		
Accuracy (%)	IRTA	98.9	97.3	99.9	99.2	99.6	100.5	99.4	97.5	97.7	100.9	100.0	100.7	99.2	100.3	101.2	100.9	100.5	99.2	101.7	99.7	1.2
10 µg/kg	RIKILT	113.9	115.6	111.4	114.0	119.6	116.6	109.6	112.6	115.1	113.7	107.0	114.0	119.2	114.8	114.1	116.9	114.2	110.8	122.6	114.5	3.6

b) PANOs

PANO:		EmNO	ErNO	EuNO	HeNO	IdNO	IrNO	JbNO	LcNO	LyNO	McNO	ReNO	RdNO	SnNO	SpNO	SvNO	TdNO	Avg	SD
Recovery (%)	IRTA	49.7	45.1	48.4	49.8	49.6	51.8	46.5	49.8	56.6	40.5	47.8	46.4	53.0	49.0	47.5	48.4	48.7	7.4
(n = 3)	RIKILT	69.6	69.3	60.8	73.4	59.7	77.3	62.0	65.5	62.5	45.2	68.9	41.5	87.3	60.8	58.0	66.9	64.3	11.1
RSD (%)	IRTA	6.5	7.8	7.4	5.2	7.1	4.7	8.7	3.1	6.3	9.2	8.5	8.1	7.5	9.7	6.2	12.0		
(n = 3)	RIKILT	3.1	9.3	3.6	0.4	2.8	3.0	2.5	3.5	3.0	3.6	3.4	1.4	27.3	1.8	3.8	3.1		
Accuracy (%)	IRTA	98.7	100.9	97.9	98.1	101.5	100.5	96.5	99.3	103.6	93.1	99.9	99.7	100.9	101.7	97.4	102.2	99.5	2.6
10 µg/kg	RIKILT	109.4	113.5	107.7	110.9	113.0	109.3	105.1	115.5	114.1	121.9	109.7	107.5	110.8	110.3	104.9	111.3	110.9	4.2

Table D.4. Results of the inter-laboratory study between RIKILT and IRTA for beef meat samples. Abbreviations are explained in Table 10.

a) PA FBs

PA FB:		Em	Er	Eu	Fs	He	Ir	Jb	Jl	Lc	Ly	Mc	Ot	Re	Rd	Sn	Sp	Sv	Sk	Td	Avg	SD
Recovery (%) (n = 3)	IRTA	48.9	65.6	53.4	52.6	54.1	53.9	63.6	50.8	54.6	56.8	65.5	55.5	56.5	62.0	55.1	57.5	55.1	58.1	61.4	56.9	2.8
RSD (%) (n = 3)	IRTA	0.6	5.3	0.8	1.9	4.6	5.7	1.4	0.9	2.4	0.6	1.8	1.3	5.7	1.2	2.8	5.4	1.5	1.6	5.8		
Accuracy (%) 5 µg/kg	IRTA	96.8	105.2	100.8	102.2	102.2	97.1	102.0	102	98.8	103.9	102.9	101.0	98.5	101.8	97.6	95.5	96.7	105.9	98.2	100.5	3.1

b) PANOs

PANO:		EmNO	ErNO	EuNO	HeNO	IdNO	IrNO	JbNO	LcNO	LyNO	McNO	ReNO	RdNO	SnNO	SpNO	SvNO	TdNO	Avg	SD
Recovery (%) (n = 3)	IRTA	48.5	43.4	56.7	55.5	54.7	46.8	42.1	45.8	56.4	44.1	45.5	41.5	42.5	43.6	40.1	41.2	46.8	1.9
RSD (%) (n = 3)	IRTA	2.2	0.0	0.6	1.3	2.9	1.0	1.1	2.1	4.0	0.6	0.7	3.0	0.2	4.1	3.0	3.3		
Accuracy (%) 5 µg/kg	IRTA	103.1	99.2	104.2	105.8	108.2	111.5	107.6	99.3	104.8	106.5	102.9	105.5	101.5	103.3	109.3	108.3	105.1	3.5

Note: The beef meat samples were not analysed by RIKILT.

Appendix E. Stability of PAs in beef, pork and poultry meat under retail storage conditions and the effect of storage on LC-MS/MS matrix effects.

Table E.1. Stability results for meat samples stored at 4 °C. Average loss or gain (%) of the free bases (PA FBs) and *N*-oxides (PANOs) in spiked (10 µg/kg) meat samples, at different time periods relative to T = 0 days (n = 3). RSD = average relative standard deviation in the measurements

Storage time (days)	Bovine meat, 10 µg/kg				Porcine meat, 10 µg/kg				Poultry meat, 10 µg/kg			
	PA FBs	RSD	PANOs	RSD	PA FBs	RSD	PANOs	RSD	PA FBs	RSD	PANOs	RSD
0	0 %	13 %	0 %	42 %	0 %	13 %	0 %	10 %	0 %	8 %	0 %	7 %
1	6 %	17 %	-41 %	84 %	-16 %	14 %	-13 %	6 %	3 %	2 %	-3 %	3 %
2	-1 %	9 %	-19 %	56 %	-8 %	7 %	-8 %	6 %	0 %	4 %	-7 %	2 %
4	2 %	14 %	-26 %	13 %	-4 %	12 %	-8 %	7 %	10 %	5 %	6 %	3 %
8	-6 %	29 %	-17 %	57 %	-15 %	12 %	-17 %	7 %	11 %	2 %	8 %	2 %

Table E.2. Stability results for meat samples stored at 4 °C. Average loss or gain (%) of the free bases (PA FBs) and *N*-oxides (PANOs) in spiked (1 µg/kg) meat samples, at different time periods relative to T = 0 days (n = 3). RSD = average relative standard deviation in the measurements

Storage time (days)	Bovine meat, 1 µg/kg				Porcine meat, 1 µg/kg				Poultry meat, 1 µg/kg			
	PA FBs	RSD	PANOs[a]	RSD[a]	PA FBs	RSD	PANOs	RSD	PA FBs	RSD	PANOs	RSD
0	0 %	11 %	0 %	47 %	0 %	8 %	0 %	5 %	0 %	4 %	0 %	3 %
1	-9 %	13 %	-15 %	71 %	6 %	6 %	-4 %	10 %	4 %	8 %	-5 %	7 %
2	-2 %	5 %	-8 %	18 %	7 %	6 %	-5 %	8 %	5 %	4 %	-5 %	5 %
4	6 %	6 %	-28 %	65 %	-5 %	7 %	-19 %	11 %	18 %	3 %	13 %	3 %
8	-20 %	13 %	-17 %	31 %	-2 %	15 %	-17 %	12 %	13 %	5 %	6 %	4 %

a. Excluding monocrotaline-*N*-oxide.

Table E.3. Averaged suppression of mass spectrometric signal (%) of the free bases (PA FBs) and *N*-oxides (PANOs) in meat samples spiked at 10 µg/kg and stored at 4 °C at different periods relative to T = 0 days (n = 3) [(a)]. SD = average standard deviation in the measurements

Storage time (days)	Bovine meat, 10 µg/kg				Porcine meat, 10 µg/kg				Poultry meat, 10 µg/kg			
	PA FBs	SD	PANOs	SD	PA FBs	SD	PANOs	SD	PA FBs	SD	PANOs	SD
0	-16 %	7 %	-73 %	12 %	-17 %	12 %	-11 %	13 %	-33 %	13 %	-22 %	12 %
1	-10 %	11 %	-86 %	8 %	-31 %	11 %	-23 %	11 %	-31 %	12 %	-25 %	11 %
2	-16 %	8 %	-79 %	9 %	-24 %	11 %	-18 %	15 %	-33 %	13 %	-29 %	12 %
4	-14 %	9 %	-81 %	9 %	-21 %	10 %	-19 %	15 %	-26 %	14 %	-16 %	13 %
8	-21 %	9 %	-79 %	8 %	-30 %	10 %	-27 %	14 %	-30 %	14 %	-15 %	13 %
Average	-15 %	9 %	-80 %	9 %	-24 %	13 %	-20 %	12 %	-29 %	13 %	-21 %	12 %

(a): For meat samples spiked at 1 µg/kg very similar results were obtained.

Appendix F. Total PA, PA free bases and PA *N*-oxides concentrations in tea infusions (in µg/L). Max, average, mean, 75th and 95th percentiles [(a)]

a) Total PA content

Type of tea	N	Maximum	Average			Median			75th Percentile			95th Percentile		
			LB	MB	UB	LB	MB	UB	LB	MB	UB	LB	MB	UB
(Herbal) teas	166	64.08	6.13	6.28	6.45	2.47	2.59	2.77	6.29	6.42	6.55	24.87	25.04	25.21
Black tea	33	54.16	7.62	7.80	7.96	1.59	1.76	1.92	5.39	5.56	5.70	(38.43)	(38.58)	(38.71)
Chamomile tea	35	18.59	3.65	3.81	3.96	1.69	1.82	1.97	5.30	5.45	5.63	(11.45)	(11.58)	(11.70)
Green tea	26	52.22	5.65	5.83	6.01	0.33	0.53	0.71	6.61	6.80	6.96	(18.29)	(18.46)	(18.61)
Mixed herbs	20	25.72	5.82	5.99	6.17	2.47	2.59	2.71	7.26	7.39	7.52	(22.46)	(22.59)	(22.70)
Peppermint tea	30	58.69	6.68	6.75	6.91	2.60	2.73	2.88	4.80	4.95	5.09	(27.74)	(27.87)	(28.03)
Rooibos tea	22	64.08	7.99	8.14	8.29	3.26	3.38	3.53	7.89	8.04	8.19	(21.93)	(22.08)	(22.24)

b) PA FBs

Type of tea	N	Maximum	Average			Median			75th Percentile			95th Percentile		
			LB	MB	UB	LB	MB	UB	LB	MB	UB	LB	MB	UB
(Herbal) teas	166	46.90	2.17	2.26	2.34	0.88	0.96	1.06	2.32	2.40	2.47	7.71	7.76	7.82
Black tea	33	6.29	1.13	1.23	1.32	0.62	0.71	0.79	1.21	1.31	1.41	(4.25)	(4.34)	(4.42)
Chamomile tea	35	4.93	1.38	1.46	1.55	0.88	0.95	1.04	2.44	2.50	2.57	(3.82)	(3.89)	(3.97)
Green tea	26	46.90	3.24	3.33	3.42	0.33	0.42	0.51	2.49	2.58	2.65	(8.64)	(8.72)	(8.79)
Mixed herbs	20	8.86	2.14	2.22	2.31	0.95	1.02	1.09	2.73	2.82	2.91	(8.40)	(8.47)	(8.58)
Peppermint tea	30	18.86	2.89	2.97	3.06	1.19	1.26	1.33	2.72	2.78	2.85	(13.50)	(13.56)	(13.65)
Rooibos tea	22	27.83	2.75	2.83	2.91	0.80	0.88	0.98	2.30	2.37	2.45	(7.74)	(7.80)	(7.85)

c) PANOs

Type of tea	N	Maximum	Average			Median			75th Percentile			95th Percentile		
			LB	MB	UB	LB	MB	UB	LB	MB	UB	LB	MB	UB
(Herbal) teas	166	49.99	3.94	4.03	4.11	1.08	1.16	1.27	4.31	4.38	4.45	14.82	14.90	14.97
Black tea	33	49.99	6.48	6.57	6.64	0.50	0.59	0.66	4.33	4.40	4.45	(34.94)	(35.00)	(35.06)
Chamomile tea	35	14.05	2.25	2.33	2.41	0.72	0.80	0.87	2.83	2.89	2.95	(9.15)	(9.21)	(9.25)
Green tea	26	13.11	2.41	2.50	2.59	0.00	0.11	0.20	4.35	4.44	4.52	(9.94)	(10.04)	(10.11)
Mixed herbs	20	24.21	3.69	3.77	3.85	0.91	0.98	1.04	4.88	4.96	5.02	(15.41)	(15.48)	(15.55)
Peppermint tea	30	39.82	3.70	3.78	3.85	1.40	1.47	1.55	2.67	2.74	2.80	(15.20)	(15.26)	(15.33)
Rooibos tea	22	36.23	5.21	5.29	5.38	2.33	2.41	2.51	6.03	6.12	6.19	(14.20)	(14.28)	(14.38)

N = number of samples, LB = lower bound, MB = middle bound, UB = upper bound.
(a): When N<60 the calculated 95th percentile is given in between brackets and should be considered as an indicative value only due to the limited number of data (EFSA, 2011).

Appendix G. Total PA, PA free bases and PA *N*-oxides concentrations in dry tea (in µg/kg). Maximum, average, mean, 75th and 95th percentiles [(a)]

d) Total PA content

Type of tea	N	Maximum	Average			Median			75th Percentile			95th Percentile		
			LB	MB	UB	LB	MB	UB	LB	MB	UB	LB	MB	UB
(Herbal) teas	166	4804.5	459.6	471.3	483.8	184.7	194.6	207.8	472.5	481.5	491.3	1865.6	1878.1	1890.7
Black tea	33	4061.5	571.6	585.1	597.3	118.6	132.2	144.2	404.5	416.7	427.7	2882.2	2893.4	2903.5
Chamomile tea	35	1394.3	273.8	285.0	297.0	124.7	135.6	148.1	397.5	409.0	422.2	859.1	868.5	877.6
Green tea	26	3916.6	423.4	437.5	450.7	24.5	39.5	53.3	496.0	509.7	522.2	1373.1	1384.4	1395.8
Mixed herbs	20	1929.2	439.4	449.4	462.5	180.2	192.0	203.0	544.8	554.4	564.0	1684.5	1693.9	1702.6
Peppermint tea	30	4401.0	496.2	506.2	518.5	195.6	203.8	215.9	360.0	370.9	382.1	2080.7	2090.1	2101.9
Rooibos tea	22	4804.5	598.5	609.2	621.9	244.0	253.1	264.8	591.8	603.3	614.1	1645.0	1656.0	1667.7

e) PA FBs

Type of tea	N	Maximum	Average			Median			75th Percentile			95th Percentile		
			LB	MB	UB	LB	MB	UB	LB	MB	UB	LB	MB	UB
(Herbal) teas	166	3517.6	163.4	169.2	175.8	67.7	72.3	79.3	175.5	179.6	184.5	578.0	582.3	586.3
Black tea	33	472.1	84.5	92.0	98.8	46.8	53.3	59.2	90.6	98.4	105.6	318.6	324.8	331.4
Chamomile tea	35	369.7	103.7	109.9	116.2	66.0	71.5	78.2	182.7	187.3	192.5	286.2	291.5	298.0
Green tea	26	3517.6	242.8	249.7	256.3	24.5	31.6	38.3	187.0	193.2	198.9	648.1	653.8	659.0
Mixed herbs	20	664.6	160.3	166.4	173.5	71.1	76.1	81.5	204.4	211.3	218.2	630.0	635.1	643.2
Peppermint tea	30	1414.3	217.0	223.0	229.7	89.2	94.2	100.1	203.8	208.7	213.5	1012.5	1017.0	1023.7
Rooibos tea	22	2087.3	206.4	212.4	218.6	59.9	65.8	73.3	172.4	177.9	184.0	580.3	584.9	589.0

f) PANOs

Type of tea	N	Maximum	Average			Median			75th Percentile			95th Percentile		
			LB	MB	UB	LB	MB	UB	LB	MB	UB	LB	MB	UB
(Herbal) teas	166	3749.6	296.2	302.1	308.0	82.4	87.3	95.4	296.2	328.7	333.6	1112.6	1117.5	1123.1
Black tea	33	3749.6	486.4	492.6	497.9	37.5	44.0	49.8	325.0	330.3	335.0	2620.2	2625.0	2629.3
Chamomile tea	35	1053.7	168.9	175.1	180.8	54.1	59.9	65.2	211.9	216.7	221.0	686.1	690.4	693.7
Green tea	26	983.5	180.6	187.8	194.4	0.0	7.9	15.0	326.0	332.7	338.7	746.6	752.1	758.5
Mixed herbs	20	1815.7	277.1	283.0	288.9	68.5	73.3	77.7	366.0	371.7	376.8	1155.6	1161.0	1166.0
Peppermint tea	30	2986.6	277.4	283.2	288.8	68.5	110.5	116.2	199.9	205.3	210.2	1139.7	1144.9	1149.5
Rooibos tea	22	2717.1	390.4	396.8	403.3	174.5	181.8	188.0	452.3	458.8	464.6	1064.7	1071.2	1078.7

N = number of samples, LB = lower bound, MB = middle bound, UB = upper bound.
(a): When N<60 the calculated 95th percentile is given in between brackets and should be considered as an indicative value only due to the limited number of data (EFSA, 2011).

Appendix H. PA concentrations (µg/L in infusion) in positive (herbal) tea samples. PA abbreviations are explained in Table 11

ID Sample	Country	Type of tea	Em	EmNO	Er	ErNO	Eu	EuNO	He	HeNO	Im	ImNO	Jb	JbNO	Lc	LcNO
FP14/0005	DE	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0006	DE	Peppermint	0.012	0.067	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.020	0.041
FP14/0007	DE	Mixed herbs	<LOD	<LOD	<LOD	<LOD	<LOD	0.073	0.040	0.029	<LOD	<LOD	<LOD	<LOD	0.064	<LOD
FP14/0008	DE	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.083	0.707	0.728	<LOD	<LOD	<LOD	<LOD
FP14/0009	DE	Chamomile	<LOD	<LOD	<LOD	<LOD	0.163	1.381	0.048	0.135	<LOD	<LOD	<LOD	<LOD	0.024	0.036
FP14/0010	DE	Chamomile	<LOD	<LOD	<LOD	<LOD	0.547	9.196	0.075	1.135	<LOD	<LOD	<LOD	<LOD	0.025	0.103
FP14/0011	DE	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0012	DE	Rooibos	<LOD	0.064	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0013	DE	Black	<LOD	<LOD	<LOD	0.160	0.160	<LOD	<LOD	<LOD	0.177	0.073	<LOD	<LOD	<LOD	<LOD
FP14/0014	DE	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0015	DE	Black	<LOD	<LOD	<LOD	<LOD	0.076	<LOD	<LOD	<LOD	0.159	0.088	<LOD	<LOD	<LOD	<LOD
FP14/0016	DE	Mixed herbs	0.244	<LOD	<LOD	<LOD	<LOD	<LOD	0.037	<LOD	0.747	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0017	DE	Peppermint	0.011	<LOD	<LOD	<LOD	<LOD	0.224	0.069	0.505	<LOD	<LOD	<LOD	<LOD	0.028	0.113
FP14/0018	DE	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.636	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0019	DE	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.413	0.317	<LOD	<LOD	<LOD	<LOD
FP14/0020	DE	Chamomile	<LOD	0.280	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.555	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0021	DE	Peppermint	0.024	<LOD	<LOD	0.087	<LOD	0.232	0.127	0.533	<LOD	<LOD	<LOD	<LOD	0.039	0.147
FP14/0022	DE	Peppermint	0.076	0.087	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.020	<LOD
FP14/0023	DE	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.452	0.043	<LOD	<LOD	<LOD	<LOD
FP14/0024	DE	Peppermint	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0025	DE	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.407	0.424	<LOD	<LOD	<LOD	<LOD
FP14/0026	DE	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.097	1.241	1.324	<LOD	<LOD	<LOD	<LOD
FP14/0027	DE	Peppermint	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0028	DE	Peppermint	<LOD	<LOD	0.067	<LOD	<LOD	0.021	<LOD	0.024	<LOD	<LOD	<LOD	<LOD	0.040	0.072
FP14/0029	DE	Camomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.083	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0030	DE	Mixed herbs	<LOD	0.053	<LOD	<LOD	<LOD	0.045	0.037	0.031	1.664	<LOD	<LOD	<LOD	0.061	<LOD
FP14/0031	DE	Mixed herbs	0.068	0.115	<LOD	<LOD	<LOD	<LOD	<LOD	0.029	0.081	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0032	DE	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.220	1.849	<LOD	0.112	<LOD	<LOD
FP14/0033	DE	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0034	DE	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.081	0.024	<LOD	<LOD	<LOD	<LOD
FP14/0035	DE	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.811	1.733	<LOD	<LOD	<LOD	<LOD
FP14/0036	DE	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.921	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0037	DE	Peppermint	0.049	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0038	DE	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.204	0.379	<LOD	<LOD	<LOD	<LOD
FP14/0040	DE	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.037	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix H, cont'd. PA concentrations (µg/L in infusion) in positive (herbal) tea samples. PA abbreviations are explained in Table 11

ID Sample	Country	Type of tea	Ly	LyNO	Re	ReNO	Sn	SnNO	Sp	SpNO	Sv	SvNO	Sk	Td
FP14/0005	DE	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0006	DE	Chamomile	<LOD	<LOD	0.164	0.508	0.216	0.580	0.596	0.236	0.048	0.051	0.045	<LOD
FP14/0007	DE	Peppermint	<LOD	<LOD	0.124	0.192	0.245	0.681	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0008	DE	Black	<LOD	<LOD	0.575	9.861	0.996	16.34	<LOD	0.083	<LOD	<LOD	<LOD	<LOD
FP14/0009	DE	Chamomile	<LOD	<LOD	<LOD	0.043	1.301	1.220	0.733	0.297	<LOD	<LOD	0.041	<LOD
FP14/0010	DE	Chamomile	<LOD	<LOD	<LOD	0.093	2.631	2.997	1.200	0.527	<LOD	<LOD	0.063	<LOD
FP14/0011	DE	Rooibos	<LOD	<LOD	0.037	0.036	0.097	0.115	<LOD	<LOD	0.035	0.027	0.048	<LOD
FP14/0012	DE	Rooibos	<LOD	<LOD	1.819	4.543	3.773	8.771	0.155	<LOD	1.979	1.128	0.060	0.043
FP14/0013	DE	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0014	DE	Rooibos	<LOD	<LOD	0.256	0.813	0.317	0.717	0.167	0.228	0.207	0.193	0.067	<LOD
FP14/0015	DE	Black	<LOD	0.027	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0016	DE	Mixed herbs	<LOD	0.072	0.628	2.595	1.685	7.204	3.071	5.073	0.853	<LOD	0.089	<LOD
FP14/0017	DE	Peppermint	<LOD	<LOD	0.065	0.228	0.148	0.416	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0018	DE	Green	<LOD	<LOD	0.820	1.189	1.328	2.024	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0019	DE	Black	<LOD	0.025	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0020	DE	Chamomile	<LOD	0.056	<LOD	0.079	0.728	1.968	0.291	0.261	<LOD	<LOD	0.032	<LOD
FP14/0021	DE	Peppermint	<LOD	<LOD	0.109	0.416	0.232	0.685	0.643	0.211	0.072	0.092	0.045	<LOD
FP14/0022	DE	Peppermint	<LOD	<LOD	0.411	0.601	0.599	1.213	2.124	0.381	0.187	0.089	0.045	<LOD
FP14/0023	DE	Chamomile	<LOD	0.147	<LOD	0.365	1.004	3.039	0.381	0.399	0.544	1.171	1.103	<LOD
FP14/0024	DE	Peppermint	<LOD	<LOD	0.173	0.205	0.344	0.467	1.169	0.273	0.120	<LOD	<LOD	<LOD
FP14/0025	DE	Black	<LOD	0.076	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0026	DE	Black	<LOD	0.103	0.616	12.10	1.043	15.24	<LOD	0.097	<LOD	<LOD	<LOD	<LOD
FP14/0027	DE	Peppermint	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0028	DE	Peppermint	<LOD	<LOD	0.360	0.397	0.529	0.892	1.659	0.545	0.220	<LOD	<LOD	<LOD
FP14/0029	DE	Chamomile	<LOD	0.032	<LOD	<LOD	0.265	0.652	0.124	0.048	<LOD	<LOD	<LOD	<LOD
FP14/0030	DE	Mixed herbs	<LOD	0.189	0.331	0.236	0.687	0.519	0.141	0.075	0.472	0.133	0.135	<LOD
FP14/0031	DE	Mixed herbs	<LOD	0.065	0.196	0.123	0.465	0.311	0.215	0.079	0.317	0.129	0.151	<LOD
FP14/0032	DE	Black	<LOD	0.147	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0033	DE	Rooibos	<LOD	<LOD	0.945	1.792	2.901	6.024	1.103	0.784	0.945	0.473	0.136	<LOD
FP14/0034	DE	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0035	DE	Black	<LOD	0.181	<LOD	0.763	0.249	1.659	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0036	DE	Chamomile	<LOD	0.027	<LOD	0.137	0.420	0.691	0.188	0.080	0.356	0.529	0.727	<LOD
FP14/0037	DE	Peppermint	<LOD	0.103	0.180	0.229	0.237	0.273	1.113	0.255	0.121	0.072	<LOD	<LOD
FP14/0038	DE	Black	<LOD	0.029	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0040	DE	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.035	<LOD

Appendix H, cont'd. PA concentrations (µg/L in infusion) in positive (herbal) tea samples. PA abbreviations are explained in Table 11

ID Sample	Country	Type of tea	Em	EmNO	Er	ErNO	Eu	EuNO	He	HeNO	Im	ImNO	Jb	JbNO	Lc	LcNO
FP14/0041	DE	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.380	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0042	DE	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0043	DE	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0044	DE	Green tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.293	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0045	DE	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0046	DE	Mixed herbs	<LOD	<LOD	<LOD	<LOD	0.011	<LOD	0.008	<LOD	<LOD	<LOD	<LOD	<LOD	0.032	<LOD
FP14/0047	DE	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.065	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0048	DE	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0049	DE	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.821	<LOD	0.135	<LOD	<LOD	<LOD
FP14/0050	DE	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0051	DE	Mixed herbs	0.016	0.043	<LOD	<LOD	<LOD	<LOD	<LOD	0.025	<LOD	<LOD	<LOD	<LOD	0.117	<LOD
FP14/0052	DE	Chamomile	<LOD	2.575	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.444	<LOD	<LOD	<LOD	0.493	0.283
FP14/0053	DE	Chamomile	<LOD	0.048	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.236	<LOD	<LOD	<LOD	0.139	0.037
FP14/0054	DE	Chamomile	<LOD	0.035	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.305	<LOD	<LOD	<LOD	0.129	<LOD
FP14/0055	DE	Peppermint	<LOD	<LOD	<LOD	<LOD	0.035	0.073	0.052	0.291	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0056	DE	Peppermint	<LOD	<LOD	<LOD	<LOD	0.029	<LOD	0.021	0.117	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0057	DE	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.527	<LOD	0.341	<LOD	<LOD	<LOD
FP14/0058	DE	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.180	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0059	DE	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0060	DE	Peppermint	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.271	<LOD	<LOD	<LOD	<LOD	0.015	<LOD
FP14/0061	DE	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.653	<LOD	<LOD	<LOD	0.247	0.125
FP14/0062	DE	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.287	0.052	<LOD	<LOD	<LOD	<LOD
FP14/0063	DE	Mixed herbs	<LOD	<LOD	<LOD	<LOD	1.961	2.496	2.777	0.092	<LOD	<LOD	<LOD	<LOD	2.216	<LOD
FP14/0064	DE	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.872	2.981	<LOD	0.063	<LOD	<LOD
FP14/0065	DE	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.143	0.089	<LOD	<LOD	<LOD	<LOD
FP14/0066	DE	Peppermint	<LOD	<LOD	<LOD	<LOD	0.013	<LOD	0.020	0.524	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0067	DE	Mixed herbs	<LOD	0.043	<LOD	<LOD	0.047	0.048	0.016	0.063	<LOD	<LOD	<LOD	<LOD	0.053	<LOD
FP14/0068	NL	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.380	0.293	<LOD	0.049	<LOD	<LOD
FP14/0069	NL	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0070	NL	Chamomile	<LOD	0.517	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.140	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0071	NL	Peppermint	0.023	<LOD	<LOD	<LOD	0.061	0.555	0.140	0.803	<LOD	<LOD	<LOD	<LOD	0.073	0.204
FP14/0072	NL	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.357	0.317	<LOD	<LOD	<LOD	<LOD
FP14/0073	NL	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	6.295	10.39	<LOD	0.475	<LOD	<LOD
FP14/0074	NL	Chamomile	<LOD	3.469	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.819	0.175	<LOD	<LOD	<LOD	<LOD
FP14/0075	NL	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix H, cont'd. PA concentrations (µg/L in infusion) in positive (herbal) tea samples. PA abbreviations are explained in Table 11

ID Sample	Country	Type of tea	Ly	LyNO	Re	ReNO	Sn	SnNO	Sp	SpNO	Sv	SvNO	Sk	Td
FP14/0041	DE	Green	<LOD	<LOD	2.399	4.955	1.975	4.608	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0042	DE	Rooibos	<LOD	<LOD	0.857	2.340	1.227	4.544	0.148	0.123	0.827	0.503	0.063	<LOD
FP14/0043	DE	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0044	DE	Green tea	<LOD	<LOD	<LOD	3.315	0.347	2.840	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0045	DE	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	0.200	0.084	0.033	<LOD	<LOD	<LOD	<LOD
FP14/0046	DE	Mixed herbs	<LOD	0.084	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.032	<LOD	0.080	<LOD
FP14/0047	DE	Rooibos	<LOD	<LOD	0.952	1.319	0.955	6.416	0.021	0.065	0.483	<LOD	0.043	<LOD
FP14/0048	DE	Rooibos	<LOD	<LOD	0.041	0.243	0.033	0.788	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0049	DE	Green	<LOD	<LOD	0.993	1.823	1.319	2.901	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0050	DE	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0051	DE	Mixed herbs	<LOD	<LOD	0.220	0.101	0.289	0.400	<LOD	0.025	0.109	<LOD	0.056	<LOD
FP14/0052	DE	Chamomile	<LOD	<LOD	0.084	0.495	0.312	1.465	0.511	0.615	0.085	<LOD	0.040	<LOD
FP14/0053	DE	Chamomile	<LOD	<LOD	<LOD	<LOD	0.300	0.396	0.141	0.060	0.059	<LOD	0.041	<LOD
FP14/0054	DE	Chamomile	<LOD	<LOD	<LOD	<LOD	0.464	0.353	0.192	0.056	0.080	<LOD	0.047	<LOD
FP14/0055	DE	Peppermint	0.051	0.272	0.181	0.240	0.125	0.416	0.429	0.291	0.060	<LOD	0.035	<LOD
FP14/0056	DE	Peppermint	0.076	0.201	0.079	<LOD	0.073	0.157	0.252	0.117	0.044	<LOD	0.035	<LOD
FP14/0057	DE	Green	<LOD	<LOD	0.273	0.049	0.465	0.233	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0058	DE	Green	<LOD	<LOD	0.307	7.540	0.475	5.575	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0059	DE	Rooibos	<LOD	<LOD	0.084	0.223	0.157	1.699	<LOD	<LOD	0.048	<LOD	0.029	<LOD
FP14/0060	DE	Peppermint	<LOD	<LOD	0.317	0.192	0.277	0.599	0.771	0.267	0.103	<LOD	0.035	<LOD
FP14/0061	DE	Chamomile	<LOD	<LOD	<LOD	0.200	0.483	0.991	0.211	0.107	0.224	0.440	0.627	<LOD
FP14/0062	DE	Black	<LOD	0.043	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0063	DE	Mixed herbs	1.048	4.168	0.092	0.061	0.073	0.149	0.167	0.092	0.031	<LOD	0.068	<LOD
FP14/0064	DE	Black	<LOD	0.427	<LOD	0.412	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0065	DE	Black	<LOD	0.024	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0066	DE	Peppermint	<LOD	<LOD	0.576	0.688	0.351	1.017	0.875	0.524	0.180	<LOD	0.048	<LOD
FP14/0067	DE	Mixed herbs	0.033	0.097	<LOD	0.085	0.117	0.287	0.129	0.063	0.059	0.027	<LOD	<LOD
FP14/0068	NL	Black	<LOD	0.069	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0069	NL	Rooibos	<LOD	0.023	0.132	0.305	0.227	0.651	<LOD	0.033	0.099	0.079	0.048	<LOD
FP14/0070	NL	Chamomile	<LOD	0.108	<LOD	<LOD	0.305	0.451	0.141	0.075	<LOD	<LOD	0.028	<LOD
FP14/0071	NL	Peppermint	<LOD	<LOD	2.049	5.067	4.628	9.789	8.428	4.200	2.184	1.607	0.060	<LOD
FP14/0072	NL	Black	<LOD	0.056	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0073	NL	Black	<LOD	0.471	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0074	NL	Chamomile	0.187	0.360	<LOD	0.491	0.713	1.837	0.608	0.885	<LOD	<LOD	0.028	<LOD
FP14/0075	NL	Rooibos	<LOD	<LOD	6.044	8.296	12.78	25.38	0.887	0.591	8.019	1.960	0.105	<LOD

Appendix H, cont'd. PA concentrations (µg/L in infusion) in positive (herbal) tea samples. PA abbreviations are explained in Table 11

ID Sample	Country	Type of tea	Em	EmNO	Er	ErNO	Eu	EuNO	He	HeNO	Im	ImNO	Jb	JbNO	Lc	LcNO
FP14/0076	NL	Mixed herbs	<LOD	<LOD	<LOD	<LOD	0.025	0.983	0.316	3.111	<LOD	<LOD	<LOD	<LOD	0.076	0.299
FP14/0077	NL	Mixed herbs	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0078	NL	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0079	NL	Black	<LOD	<LOD	<LOD	0.141	<LOD	<LOD	<LOD	<LOD	1.244	2.551	<LOD	<LOD	<LOD	<LOD
FP14/0080	NL	Mixed herbs	<LOD	<LOD	<LOD	<LOD	0.037	0.527	0.355	1.515	<LOD	<LOD	<LOD	<LOD	0.200	0.669
FP14/0081	NL	Green	<LOD	<LOD	<LOD	<LOD	0.049	<LOD	<LOD	<LOD	0.173	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0082	NL	Green	<LOD	<LOD	<LOD	<LOD	0.044	<LOD	<LOD	<LOD	0.071	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0083	NL	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.735	0.805	<LOD	<LOD	<LOD	<LOD
FP14/0085	NL	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.313	0.539	<LOD	<LOD	<LOD	<LOD
FP14/0086	NL	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.105	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0087	NL	Peppermint	<LOD	<LOD	<LOD	<LOD	<LOD	0.131	0.027	0.227	<LOD	<LOD	<LOD	<LOD	0.052	0.149
FP14/0088	NL	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4.221	0.145	<LOD	<LOD	<LOD	<LOD
FP14/0089	NL	Peppermint	<LOD	<LOD	<LOD	<LOD	<LOD	0.121	0.013	0.065	<LOD	<LOD	<LOD	<LOD	0.040	<LOD
FP14/0090	NL	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0091	NL	Peppermint	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0092	NL	Green	<LOD	<LOD	<LOD	<LOD	0.071	<LOD	0.108	<LOD	0.136	<LOD	0.129	<LOD	<LOD	<LOD
FP14/0093	NL	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0094	NL	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.049	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0095	NL	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0096	NL	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0097	NL	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0098	NL	Mixed herbs	<LOD	<LOD	<LOD	<LOD	0.352	12.45	0.920	8.227	<LOD	<LOD	<LOD	<LOD	0.239	3.537
FP14/0099	NL	Peppermint	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0300	ES	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.055	0.205	0.691	0.717	<LOD	<LOD	<LOD	<LOD
FP14/0301	ES	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.957	1.501	<LOD	<LOD	<LOD	<LOD
FP14/0302	ES	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.053	0.213	0.712	0.756	<LOD	<LOD	<LOD	<LOD
FP14/0303	ES	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0304	ES	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0305	ES	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	5.513	<LOD	0.309	<LOD	<LOD	0.173
FP14/0306	ES	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.147	0.093	1.804	<LOD	0.335	<LOD	<LOD	<LOD
FP14/0307	ES	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.031	3.816	<LOD	0.297	<LOD	<LOD	<LOD
FP14/0308	ES	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0309	ES	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0310	ES	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0311	ES	Rooibos	<LOD	0.053	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix H, cont'd. PA concentrations (µg/L in infusion) in positive (herbal) tea samples. PA abbreviations are explained in Table 11

ID Sample	Country	Type of tea	Ly	LyNO	Re	ReNO	Sn	SnNO	Sp	SpNO	Sv	SvNO	Sk	Td
FP14/0076	NL	Mixed herbs	<LOD	<LOD	0.099	0.144	0.228	0.421	0.248	0.244	0.117	<LOD	<LOD	<LOD
FP14/0077	NL	Mixed herbs	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0078	NL	Rooibos	<LOD	<LOD	0.247	0.735	0.540	2.451	<LOD	0.068	0.252	0.207	0.056	<LOD
FP14/0079	NL	Black	<LOD	0.533	0.101	2.000	0.329	3.800	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0080	NL	Mixed herbs	<LOD	<LOD	<LOD	<LOD	<LOD	0.147	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0081	NL	Green	<LOD	<LOD	0.267	0.124	0.517	0.624	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0082	NL	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0083	NL	Black	<LOD	0.043	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0085	NL	Black	<LOD	0.097	0.075	0.461	0.236	0.981	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0086	NL	Green	<LOD	<LOD	1.833	3.247	2.016	3.912	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0087	NL	Peppermint	<LOD	<LOD	<LOD	<LOD	0.068	0.065	0.187	0.059	0.049	<LOD	<LOD	<LOD
FP14/0088	NL	Chamomile	0.708	0.132	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0089	NL	Peppermint	<LOD	<LOD	0.136	0.244	0.228	0.520	0.539	0.283	0.147	0.128	<LOD	<LOD
FP14/0090	NL	Chamomile	<LOD	<LOD	<LOD	0.099	1.977	2.628	1.005	0.276	0.221	<LOD	0.112	<LOD
FP14/0091	NL	Peppermint	0.033	<LOD	<LOD	<LOD	<LOD	0.048	0.075	0.035	<LOD	<LOD	<LOD	<LOD
FP14/0092	NL	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0093	NL	Rooibos	<LOD	<LOD	0.464	1.448	0.621	2.632	0.107	0.087	0.543	0.313	0.060	<LOD
FP14/0094	NL	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	0.091	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0095	NL	Rooibos	<LOD	0.020	0.112	0.236	0.227	0.735	<LOD	0.031	0.088	0.085	0.048	<LOD
FP14/0096	NL	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0097	NL	Chamomile	<LOD	<LOD	<LOD	0.081	<LOD	0.251	0.244	0.171	0.077	<LOD	<LOD	<LOD
FP14/0098	NL	Mixed herbs	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0099	NL	Peppermint	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.047	<LOD	<LOD	<LOD	<LOD
FP14/0300	ES	Black	<LOD	<LOD	1.339	19.84	2.297	22.92	<LOD	0.205	<LOD	<LOD	<LOD	<LOD
FP14/0301	ES	Black	<LOD	0.185	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0302	ES	Black	<LOD	<LOD	1.197	21.96	2.196	26.85	<LOD	0.213	<LOD	<LOD	<LOD	<LOD
FP14/0303	ES	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0304	ES	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0305	ES	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.081	<LOD
FP14/0306	ES	Green	<LOD	<LOD	19.32	2.724	24.53	2.411	0.693	0.093	<LOD	<LOD	0.076	<LOD
FP14/0307	ES	Green	<LOD	<LOD	1.713	3.055	3.652	6.969	<LOD	0.031	<LOD	<LOD	0.073	<LOD
FP14/0308	ES	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0309	ES	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0310	ES	Rooibos	<LOD	<LOD	0.417	1.143	1.139	3.940	0.285	0.201	0.673	0.375	0.049	<LOD
FP14/0311	ES	Rooibos	<LOD	<LOD	0.309	0.588	0.797	2.364	0.123	0.013	0.573	0.211	0.039	<LOD

Appendix H, cont'd. PA concentrations (µg/L in infusion) in positive (herbal) tea samples. PA abbreviations are explained in Table 11

ID Sample	Country	Type of tea	Em	EmNO	Er	ErNO	Eu	EuNO	He	HeNO	Im	ImNO	Jb	JbNO	Lc	LcNO
FP14/0312	ES	Rooibos	<LOD	0.068	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0313	ES	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.284	1.481	<LOD	<LOD	<LOD	<LOD
FP14/0314	ES	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0315	ES	Chamomile	0.081	<LOD	<LOD	<LOD	0.016	0.157	0.067	0.172	0.047	<LOD	<LOD	<LOD	0.081	0.165
FP14/0316	ES	Chamomile	0.319	0.160	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.591	0.068	<LOD	<LOD	<LOD	<LOD
FP14/0317	ES	Chamomile	0.075	<LOD	<LOD	<LOD	<LOD	0.105	0.053	0.116	0.032	<LOD	<LOD	<LOD	0.073	0.119
FP14/0318	ES	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.020	<LOD	<LOD	<LOD	<LOD	0.061	<LOD
FP14/0319	ES	Chamomile	<LOD	0.093	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.264	0.553	<LOD	<LOD	<LOD	<LOD
FP14/0320	ES	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0321	ES	Peppermint	0.027	<LOD	<LOD	<LOD	0.147	1.092	0.031	0.256	<LOD	<LOD	<LOD	<LOD	0.181	1.379
FP14/0322	ES	Peppermint	0.029	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.016	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0323	ES	Peppermint	<LOD	<LOD	0.083	0.056	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0324	ES	Peppermint	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.028	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0325	ES	Peppermint	<LOD	0.085	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0326	ES	Peppermint	<LOD	<LOD	<LOD	<LOD	0.011	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0327	ES	Mixed herbs	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0328	ES	Mixed herbs	<LOD	<LOD	<LOD	<LOD	0.023	<LOD	0.024	0.461	<LOD	<LOD	<LOD	<LOD	0.057	<LOD
FP14/0329	ES	Mixed herbs	<LOD	<LOD	<LOD	<LOD	0.011	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.453	<LOD
FP14/0330	ES	Mixed herbs	<LOD	<LOD	<LOD	<LOD	<LOD	0.032	<LOD	0.055	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0331	FR	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.975	3.921	<LOD	0.068	<LOD	<LOD
FP14/0332	FR	Black	<LOD	<LOD	<LOD	0.235	<LOD	<LOD	<LOD	<LOD	1.135	1.265	<LOD	0.280	<LOD	<LOD
FP14/0333	FR	Black	<LOD	<LOD	<LOD	<LOD	0.145	<LOD	<LOD	<LOD	0.101	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0334	FR	Black	<LOD	<LOD	<LOD	<LOD	0.143	<LOD	<LOD	<LOD	0.133	0.019	<LOD	<LOD	<LOD	<LOD
FP14/0335	FR	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.072	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0336	FR	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.043	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0337	FR	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.011	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0338	FR	Chamomile	0.137	0.112	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.227	0.337	<LOD	<LOD	<LOD	<LOD
FP14/0339	FR	Chamomile	<LOD	9.855	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.377	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0340	FR	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0341	FR	Peppermint	0.027	<LOD	<LOD	<LOD	0.027	0.104	0.099	0.309	0.037	<LOD	<LOD	<LOD	0.060	0.133
FP14/0342	FR	Peppermint	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.129	<LOD
FP14/0343	FR	Peppermint	<LOD	<LOD	<LOD	<LOD	1.856	2.499	2.108	<LOD	<LOD	<LOD	<LOD	<LOD	0.068	<LOD
FP14/0344	FR	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.055	<LOD	<LOD	<LOD	<LOD	0.012
FP14/0345	FR	Mixed herbs	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.388	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0346	FR	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.660	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix H, cont'd. PA concentrations (µg/L in infusion) in positive (herbal) tea samples. PA abbreviations are explained in Table 11

ID Sample	Country	Type of tea	Ly	LyNO	Re	ReNO	Sn	SnNO	Sp	SpNO	Sv	SvNO	Sk	Td
FP14/0312	ES	Rooibos	<LOD	0.089	0.167	0.509	0.312	1.191	<LOD	0.023	0.233	0.141	0.032	<LOD
FP14/0313	ES	Rooibos	<LOD	0.264	<LOD	0.020	0.063	0.249	<LOD	<LOD	<LOD	0.017	0.029	<LOD
FP14/0314	ES	Rooibos	<LOD	<LOD	0.089	0.151	0.271	0.604	<LOD	<LOD	0.159	0.056	0.029	<LOD
FP14/0315	ES	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	0.149	<LOD	<LOD	0.083	0.077	0.100	<LOD
FP14/0316	ES	Chamomile	0.077	0.085	<LOD	0.099	0.865	1.239	0.383	0.219	0.157	<LOD	0.091	0.077
FP14/0317	ES	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	0.121	<LOD	<LOD	0.076	0.073	0.092	<LOD
FP14/0318	ES	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	0.133	<LOD	<LOD	0.093	0.076	0.084	<LOD
FP14/0319	ES	Chamomile	0.156	0.205	<LOD	0.181	0.445	1.056	0.228	0.177	0.201	0.379	0.296	0.156
FP14/0320	ES	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0321	ES	Peppermint	<LOD	<LOD	0.092	0.173	0.124	0.352	0.312	0.183	0.071	0.063	<LOD	<LOD
FP14/0322	ES	Peppermint	<LOD	<LOD	0.108	0.109	0.319	0.248	0.493	0.119	0.156	<LOD	<LOD	<LOD
FP14/0323	ES	Peppermint	<LOD	<LOD	0.284	0.519	0.665	1.097	1.016	0.513	0.199	<LOD	<LOD	<LOD
FP14/0324	ES	Peppermint	<LOD	<LOD	0.015	<LOD	0.455	0.215	0.560	0.028	1.215	<LOD	<LOD	<LOD
FP14/0325	ES	Peppermint	<LOD	<LOD	<LOD	<LOD	<LOD	0.013	0.091	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0326	ES	Peppermint	<LOD	<LOD	<LOD	3.953	4.116	13.11	13.49	20.86	1.244	1.909	<LOD	<LOD
FP14/0327	ES	Rooibos	<LOD	<LOD	0.103	0.192	0.077	0.547	0.021	<LOD	0.049	0.027	0.032	<LOD
FP14/0328	ES	Mixed herbs	<LOD	0.072	0.769	0.953	1.480	4.603	0.397	0.461	0.828	<LOD	0.063	<LOD
FP14/0329	ES	Mixed herbs	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0330	ES	Mixed herbs	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0331	FR	Black	<LOD	0.353	<LOD	1.076	0.107	1.657	<LOD	<LOD	0.123	1.661	<LOD	<LOD
FP14/0332	FR	Black	<LOD	0.187	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0333	FR	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0334	FR	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0335	FR	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0336	FR	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0337	FR	Rooibos	<LOD	<LOD	0.115	0.395	0.099	1.393	<LOD	<LOD	0.047	<LOD	0.036	<LOD
FP14/0338	FR	Chamomile	0.080	<LOD	<LOD	0.481	0.300	0.532	0.184	0.181	0.088	<LOD	0.087	0.080
FP14/0339	FR	Chamomile	0.076	0.061	<LOD	0.599	0.716	2.227	0.877	0.956	0.160	<LOD	0.084	0.076
FP14/0340	FR	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0341	FR	Peppermint	0.073	0.036	0.920	0.840	3.428	2.183	3.227	0.895	0.652	<LOD	<LOD	0.073
FP14/0342	FR	Peppermint	<LOD	<LOD	0.131	0.140	0.105	0.155	0.136	0.048	0.053	0.047	<LOD	<LOD
FP14/0343	FR	Peppermint	2.227	3.067	<LOD	<LOD	<LOD	<LOD	0.100	<LOD	<LOD	<LOD	<LOD	2.227
FP14/0344	FR	Chamomile	0.036	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.036
FP14/0345	FR	Mixed herbs	<LOD	<LOD	0.860	0.995	1.133	1.003	4.737	1.388	2.131	<LOD	<LOD	<LOD
FP14/0346	FR	Chamomile	<LOD	<LOD	0.340	0.257	0.453	0.487	0.625	0.660	0.203	<LOD	<LOD	<LOD

Appendix H, cont'd. PA concentrations (µg/L in infusion) in positive (herbal) tea samples. PA abbreviations are explained in Table 11

ID Sample	Country	Type of tea	Em	EmNO	Er	ErNO	Eu	EuNO	He	HeNO	Im	ImNO	Jb	JbNO	Lc	LcNO
FP14/0347	IT	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.032	<LOD	<LOD	<LOD	<LOD
FP14/0348	IT	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.801	1.653	<LOD	<LOD	<LOD	<LOD
FP14/0349	IT	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.957	2.283	<LOD	<LOD	<LOD	<LOD
FP14/0350	IT	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.145	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0351	IT	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.209	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0352	IT	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.041	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0353	IT	Fennel	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0354	IT	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.369	<LOD	<LOD	<LOD	0.065	<LOD
FP14/0355	IT	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0356	IT	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0357	IT	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0358	IT	Rooibos	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.039	<LOD	<LOD	<LOD	0.076	0.117
FP14/0359	IT	Mixed herbs	0.196	0.727	<LOD	<LOD	<LOD	<LOD	0.039	0.213	0.019	<LOD	<LOD	<LOD	0.027	0.112
FP14/0360	IT	Mixed herbs	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0361	IT	Fennel	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0362	IT	Chamomile	<LOD	<LOD	<LOD	<LOD	0.032	<LOD	0.009	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0363	IT	Peppermint	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.689	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0364	IT	Peppermint	<LOD	0.325	<LOD	<LOD	<LOD	<LOD	<LOD	0.085	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0365	IT	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0366	IT	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.028	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0367	EL	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.049	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0368	EL	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0369	EL	Chamomile	<LOD	4.365	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.697	<LOD	<LOD	<LOD	0.636	0.099
FP14/0370	EL	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.123	0.069	<LOD	<LOD	<LOD	<LOD
FP14/0371	EL	Black	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.121	0.061	<LOD	<LOD	<LOD	<LOD
FP14/0372	EL	Peppermint	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.793	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0373	EL	Peppermint	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0374	EL	Mixed herbs	<LOD	<LOD	<LOD	<LOD	0.032	<LOD	0.037	<LOD	0.033	<LOD	<LOD	<LOD	0.051	<LOD

Appendix H, cont'd. PA concentrations (µg/L in infusion) in positive (herbal) tea samples. PA abbreviations are explained in Table 11

ID Sample	Country	Type of tea	Ly	LyNO	Re	ReNO	Sn	SnNO	Sp	SpNO	Sv	SvNO	Sk	Td
FP14/0347	IT	Black	<LOD	0.020	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0348	IT	Green	<LOD	0.097	0.184	3.639	0.416	2.457	<LOD	<LOD	<LOD	5.093	<LOD	<LOD
FP14/0349	IT	Black tea	<LOD	0.135	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0350	IT	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0351	IT	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0352	IT	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0353	IT	Fennel	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0354	IT	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0355	IT	Chamomile	<LOD	<LOD	<LOD	0.065	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0356	IT	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0357	IT	Rooibos	<LOD	<LOD	0.108	0.413	0.559	5.741	<LOD	<LOD	0.069	<LOD	0.049	<LOD
FP14/0358	IT	Rooibos	<LOD	<LOD	0.177	0.596	0.387	1.907	0.027	0.033	0.145	<LOD	0.037	<LOD
FP14/0359	IT	Mixed herbs	0.068	<LOD	<LOD	0.165	0.527	0.771	0.349	0.191	0.071	0.047	<LOD	<LOD
FP14/0360	IT	Mixed herbs	<LOD	<LOD	<LOD	0.035	<LOD	0.099	0.025	0.056	<LOD	0.012	0.028	<LOD
FP14/0361	IT	Fennel	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0362	IT	Chamomile	<LOD	<LOD	<LOD	0.083	1.773	3.892	0.347	0.257	0.193	<LOD	0.147	<LOD
FP14/0363	IT	Peppermint	<LOD	<LOD	0.700	1.543	0.563	1.677	3.635	1.689	0.927	<LOD	<LOD	<LOD
FP14/0364	IT	Peppermint	<LOD	<LOD	0.013	<LOD	<LOD	0.197	0.161	0.085	<LOD	<LOD	<LOD	<LOD
FP14/0365	IT	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0366	IT	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0367	EL	Green	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0368	EL	Chamomile	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0369	EL	Chamomile	<LOD	<LOD	<LOD	0.117	0.125	0.751	0.053	0.129	<LOD	<LOD	0.016	<LOD
FP14/0370	EL	Black	<LOD	0.020	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0371	EL	Black	<LOD	0.028	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0372	EL	Peppermint	<LOD	<LOD	0.233	0.568	0.600	2.424	1.205	0.793	0.936	<LOD	<LOD	<LOD
FP14/0373	EL	Peppermint	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP14/0374	EL	Mixed herbs	0.029	0.083	0.076	0.061	0.124	0.208	<LOD	<LOD	0.060	0.223	0.033	<LOD

Appendix I. PA concentrations (µg/kg) in positive (herbal) food supplement samples. PA abbreviations are explained in Table 11

ID sample	Country	Type of supplement	Em	EmNO	Er	ErNO	Eu	EuNO	He	HeNO	Im	ImNO	Jb	JbNO	Lc	LcNO
FP-14-0701	DE	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0702	DE	Plant extract formula	375.2	620.8	12.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0707	DE	Plant extract formula	95.0	321.8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0708	DE	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0709	DE	Plant extract formula	348.2	575.5	14.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0716	DE	Plant extract formula	<LOD	<LOD	40.0	<LOD	103.5	<LOD	79.4	<LOD	<LOD	<LOD	<LOD	<LOD	37.3	<LOD
FP-14-0717	DE	Plant extract formula	13.7	<LOD	359.7	<LOD	20.7	<LOD	7.5	<LOD	100.1	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0718	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	6.1	1.5	<LOD	<LOD	<LOD	<LOD
FP-14-0721	DE	Supplements containing special fatty acids	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0722	DE	Pollen-based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.9	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0723	DE	Pollen-based supplement	285.1	618.0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	111.3	91.9	<LOD	<LOD	<LOD	<LOD
FP-14-0727	DE	Pollen-based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	10.1	25.2	<LOD	<LOD	<LOD	<LOD
FP-14-0728	DE	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.0	<LOD	21.4	0.8	<LOD	<LOD	<LOD	<LOD
FP-14-0731	DE	Pollen-based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0734	DE	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0735	DE	Pollen-based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.1	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0736	DE	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0737	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	21.9	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0738	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0739	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0740	DE	Pollen-based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0742	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	10.8	2.6	4.1	<LOD	<LOD	29.6	<LOD	<LOD	10.9	<LOD
FP-14-0744	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	11.4	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0747	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	17.2	<LOD	<LOD	<LOD	<LOD
FP-14-0748	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	414294	<LOD	<LOD	<LOD	<LOD
FP-14-0749	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	795.6	4758.4	<LOD	<LOD	<LOD	<LOD
FP-14-0750	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	94.6	126.5	<LOD	<LOD	<LOD	<LOD
FP-14-0751	DE	Dietary supplements	<LOD	<LOD	39.0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0752	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	22.8	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0754	DE	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0755	DE	Dietary supplements	<LOD	<LOD	38.1	<LOD	<LOD	<LOD	<LOD	<LOD	2.3	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0757	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.1	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0758	DE	Pollen-based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.5	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0759	DE	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4.5	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0760	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	12.4	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix I, cont'd. PA concentrations (µg/kg) in positive (herbal) food supplement samples. PA abbreviations are explained in Table 11

ID sample	Country	Type of supplement	Ly	LyNO	Mc	McNO	Re	ReNO	Sn	SnNO	Sp	SpNO	Sv	SvNO	Sk	Td
FP-14-0701	DE	Plant extract formula	120.1	22.0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0702	DE	Plant extract formula	2.2	16.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0707	DE	Plant extract formula	39.4	56.8	<LOD	<LOD	48.6	55.5	18.4	12.4	30.6	<LOD	27.8	4.0	12.2	<LOD
FP-14-0708	DE	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	9.0	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0709	DE	Plant extract formula	<LOD	17.2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0716	DE	Plant extract formula	<LOD	<LOD	<LOD	<LOD	21.9	<LOD	43.1	<LOD	59.1	<LOD	6.1	<LOD	<LOD	<LOD
FP-14-0717	DE	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0718	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0721	DE	Supplements containing special fatty acids	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0722	DE	Pollen-based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0723	DE	Pollen-based supplement	43.5	33.2	<LOD	<LOD	<LOD	<LOD	18.7	61.5	<LOD	<LOD	7.7	46.3	0.4	<LOD
FP-14-0727	DE	Pollen-based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	12.6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0728	DE	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0731	DE	Pollen-based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	8.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0734	DE	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0735	DE	Pollen-based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0736	DE	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0737	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.4	<LOD
FP-14-0738	DE	Dietary supplements	5751.7	2736.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0739	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	6.9	20.8	26.4	26.1	46.8	24.2	5.8	<LOD	<LOD	<LOD
FP-14-0740	DE	Pollen-based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0742	DE	Dietary supplements	104.4	<LOD	11.6	15.9	<LOD	<LOD	<LOD	<LOD	<LOD	38.3	<LOD	<LOD	8.6	<LOD
FP-14-0744	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0747	DE	Dietary supplements	5.9	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0748	DE	Dietary supplements	604718	58536	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0749	DE	Dietary supplements	1916.9	10139	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0750	DE	Dietary supplements	7.6	28.2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0751	DE	Dietary supplements	19.3	43.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0752	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0754	DE	Plant extract formula	<LOD	1261.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0755	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0757	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0758	DE	Pollen-based supplement	2.5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.8	<LOD
FP-14-0759	DE	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0760	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	13.3	<LOD	5.3	11.3	10.5	<LOD	<LOD	<LOD	<LOD

Appendix I, cont'd. PA concentrations (µg/kg) in positive (herbal) food supplement samples. PA abbreviations are explained in Table 11

ID sample	Country	Type of supplement	Em	EmNO	Er	ErNO	Eu	EuNO	He	HeNO	Im	ImNO	Jb	JbNO	Lc	LcNO
FP-14-0762	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	6.2	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0763	DE	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0764	DE	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	16.4	14.3	<LOD	<LOD	<LOD	<LOD
FP-14-0767	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	7.0	<LOD	47.7	<LOD	9.1	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0772	NL	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	219.2	7.2	<LOD	<LOD	<LOD	<LOD
FP-14-0774	NL	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	8.8	2.2	<LOD	<LOD	<LOD	<LOD
FP-14-0775	NL	Dietary supplements	1166.5	40.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	23.3	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0776	NL	Pollen-based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0777	NL	Dietary supplements	35.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	372.3	6.5	<LOD	<LOD	<LOD	<LOD
FP-14-0778	NL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	43.2	35.8	<LOD	<LOD	<LOD	<LOD
FP-14-0779	NL	Pollen-based supplement	81.0	35.7	43.1	233.1	<LOD	<LOD	<LOD	<LOD	97.9	531.7	<LOD	<LOD	<LOD	<LOD
FP-14-0780	NL	Pollen-based supplement	30.1	69.2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	14.1	8.4	<LOD	<LOD	<LOD	<LOD
FP-14-0781	NL	Dietary supplements	<LOD	<LOD	<LOD	<LOD	2.2	1.3	0.3	<LOD	126.7	147.9	<LOD	<LOD	<LOD	<LOD
FP-14-0783	NL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.8	<LOD	<LOD	<LOD	<LOD
FP-14-0784	NL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	142.4	23.5	87.1	13.7	48.9	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0785	NL	Dietary supplements	624.2	59.8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	61.0	2.2	<LOD	<LOD	<LOD	<LOD
FP-14-0786	NL	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	22.4	2.4	<LOD	<LOD	<LOD	<LOD
FP-14-0787	NL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	33.4	17.5	<LOD	<LOD	<LOD	<LOD
FP-14-0788	NL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	13.0	2.8	<LOD	<LOD	<LOD	<LOD
FP-14-0791	NL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0794	NL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0796	NL	Pollen-based supplement	22.9	274.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4.8	8.0	<LOD	<LOD	<LOD	<LOD
FP-14-0798	NL	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	330.7	32.7	<LOD	<LOD	<LOD	<LOD
FP-14-0799	NL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	31.5	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0800	NL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	37.1	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0803	NL	Pollen-based supplement	161.9	820.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4.9	6.1	<LOD	<LOD	<LOD	<LOD
FP-14-0807	FR	Pollen-based supplement	1366.4	356.8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	70.1	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0808	FR	Dietary supplements	<LOD	42.9	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3697.5	7735.0	<LOD	<LOD	<LOD	<LOD
FP-14-0809	FR	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	836804	<LOD	<LOD	<LOD	<LOD
FP-14-0810	NL	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	981.3	<LOD	<LOD	<LOD	<LOD
FP-14-0812	ES	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	908.4	107.0	<LOD	<LOD	<LOD	<LOD
FP-14-0813	ES	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	25.1	39.8	<LOD	<LOD	<LOD	<LOD
FP-14-0815	ES	Plant extract formula	<LOD	<LOD	<LOD	<LOD	4.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0817	ES	Plant extract formula	<LOD	<LOD	<LOD	<LOD	10.6	<LOD	14.3	<LOD	<LOD	<LOD	<LOD	<LOD	5.4	<LOD
FP-14-0821	ES	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	19.6	10.5	<LOD	<LOD	<LOD	<LOD
FP-14-0824	ES	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	174.8	65.3	<LOD	<LOD	<LOD	<LOD

Appendix I, cont'd. PA concentrations (µg/kg) in positive (herbal) food supplement samples. PA abbreviations are explained in Table 11

ID sample	Country	Type of supplements	Ly	LyNO	Mc	McNO	Re	ReNO	Sn	SnNO	Sp	SpNO	Sv	SvNO	Sk	Td
FP-14-0762	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0763	DE	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	36.9	<LOD
FP-14-0764	DE	Plant extract formula	38.6	<LOD	<LOD	<LOD	<LOD	30.6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0767	DE	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0772	NL	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0774	NL	Dietary supplements	0.6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0775	NL	Dietary supplements	57.2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0776	NL	Pollen-based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.6	4.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0777	NL	Dietary supplements	224.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0778	NL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	16.1	2.4	21.2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0779	NL	Pollen-based supplement	<LOD	30.2	<LOD	<LOD	<LOD	<LOD	23.6	120.9	22.9	105.8	<LOD	<LOD	<LOD	<LOD
FP-14-0780	NL	Pollen-based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0781	NL	Dietary supplements	<LOD	0.5	30.4	88.8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	13.7
FP-14-0783	NL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0784	NL	Plant extract formula	<LOD	<LOD	328.0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	99.5
FP-14-0785	NL	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0786	NL	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0787	NL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0788	NL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0791	NL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.5	<LOD	9.1	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0794	NL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	97.7	<LOD	101.3	<LOD	196.2	<LOD	48.4	<LOD	<LOD	<LOD
FP-14-0796	NL	Pollen-based supplement	9.3	32.6	<LOD	<LOD	<LOD	<LOD	<LOD	5.0	<LOD	<LOD	<LOD	1.4	<LOD	<LOD
FP-14-0798	NL	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0799	NL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	27.6	60.0	12.2	160.6	<LOD	35.8	<LOD	<LOD	<LOD
FP-14-0800	NL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0803	NL	Pollen-based supplement	13.3	25.5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0807	FR	Pollen-based supplement	116.5	1.6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0808	FR	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	532.7	2191.3	357.4	<LOD	<LOD	<LOD
FP-14-0809	FR	Dietary supplements	1467654	105817	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0810	NL	Dietary supplements	868.7	75.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0812	ES	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	5.7	<LOD
FP-14-0813	ES	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	12.5	<LOD	<LOD	<LOD	<LOD	5.2	<LOD
FP-14-0815	ES	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0817	ES	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0821	ES	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0824	ES	Dietary supplements	283.2	65.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix I, cont'd. PA concentrations (µg/kg) in positive (herbal) food supplement samples. PA abbreviations are explained in Table 11

ID sample	Country	Type of supplement	Em	EmNO	Er	ErNO	Eu	EuNO	He	HeNO	Im	ImNO	Jb	JbNO	Lc	LcNO
FP-14-0826	ES	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0828	ES	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0829	ES	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0830	ES	Dietary supplements	<LOD	14.4	<LOD	<LOD	14.4	31.7	<LOD	<LOD	4.8	13.5	<LOD	<LOD	<LOD	<LOD
FP-14-0831	ES	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	27.7	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0837	ES	Pollen based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0838	ES	Pollen based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0839	ES	Pollen-based supplement	195.1	253.9	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	6.6	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0840	ES	Pollen-based supplement	44.6	247.2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.8	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0843	IT	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0846	IT	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0847	IT	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0850	IT	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0851	IT	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4.2	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0855	IT	Pollen-based supplement	6.7	18.9	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0856	FR	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	5.5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0859	FR	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0860	FR	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0861	FR	Dietary supplements	7.2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0862	FR	Plant extract formula	5358.6	779.0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0863	FR	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	174.5	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0864	FR	Plant extract formula	<LOD	<LOD	<LOD	<LOD	5.0	<LOD	4.5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0869	FR	Pollen-based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	14.7	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0871	EL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	42.0	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0873	EL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	8.0	1.0	3.0	<LOD	13.9	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0874	EL	Plant extract formula	689.2	130.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	69.8	8.3	<LOD	<LOD	<LOD	<LOD
FP-14-0876	EL	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0877	EL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	108.2	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0881	EL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.5	<LOD
FP-14-0884	ES	Plant extract formula	<LOD	<LOD	<LOD	<LOD	156.2	39.8	4.5	<LOD	97.8	688.2	<LOD	<LOD	20.9	<LOD
FP-14-0898	ES	Plant extract formula	<LOD	<LOD	<LOD	<LOD	4.8	3.4	2.1	<LOD	<LOD	62.1	<LOD	<LOD	<LOD	<LOD

Appendix I, cont'd. PA concentrations (µg/kg) in positive (herbal) food supplement samples. PA abbreviations are explained in Table 11

ID sample	Country	Type of supplement	Ly	LyNO	Mc	McNO	Re	ReNO	Sn	SnNO	Sp	SpNO	Sv	SvNO	Sk	Td
FP-14-0826	ES	Dietary supplements	<LOD	<LOD	<LOD	<LOD	132.1	<LOD	290.1	8.4	303.0	<LOD	11.4	<LOD	<LOD	<LOD
FP-14-0828	ES	Plant extract formula	18.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0829	ES	Dietary supplements	<LOD	6.5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0830	ES	Dietary supplements	<LOD	1.0	215.2	955.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	50.0
FP-14-0831	ES	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	39.6	<LOD	<LOD	<LOD	<LOD	<LOD	404.3	<LOD
FP-14-0837	ES	Pollen based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.9	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0838	ES	Pollen based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0839	ES	Pollen-based supplement	42.9	17.2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0840	ES	Pollen-based supplement	2.0	6.6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0843	IT	Dietary supplements	506.2	50.2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0846	IT	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.9	<LOD	11.3	<LOD	10.1	<LOD	<LOD	<LOD
FP-14-0847	IT	Dietary supplements	81.2	21.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0850	IT	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4.0	<LOD	<LOD	<LOD
FP-14-0851	IT	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0855	IT	Pollen-based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	9.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0856	FR	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0859	FR	Dietary supplements	<LOD	<LOD	<LOD	<LOD	18.6	<LOD	25.4	<LOD	90.3	<LOD	13.0	<LOD	<LOD	<LOD
FP-14-0860	FR	Plant extract formula	56.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.6	<LOD
FP-14-0861	FR	Dietary supplements	111.4	2.8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	11.5	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0862	FR	Plant extract formula	22.2	16.0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0863	FR	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.8	<LOD
FP-14-0864	FR	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0869	FR	Pollen-based supplement	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0871	EL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0873	EL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0874	EL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0876	EL	Dietary supplements	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	26.2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0877	EL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0881	EL	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0884	ES	Plant extract formula	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	10.0	5.5	<LOD	<LOD	<LOD
FP-14-0898	ES	Plant extract formula	68.6	5.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	8.5	<LOD	15614	<LOD

Appendix J. PA concentrations (µg/L in infusion) in positive (herbal) food supplement samples to be used as infusion. PA abbreviations are explained in Table 11

ID sample	Country	Type of supplement	Em	EmNO	Er	ErNO	Eu	EuNO	He	HeNO	Im	ImNO	Jb	JbNO	Lc	LcNO
FP-14-0792	NL	Tea and herbs for infusions (Solid)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.22	2.90	<LOD	<LOD	<LOD	<LOD
FP-14-0793	NL	Tea and herbs for infusions (Solid)	<LOD	<LOD	0.35	<LOD	<LOD	<LOD	<LOD	<LOD	4.87	8.63	<LOD	<LOD	<LOD	<LOD
FP-14-0804	NL	Tea and herbs for infusions (Solid)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.20	0.16	<LOD	<LOD	<LOD	<LOD
FP-14-0805	NL	Tea and herbs for infusions (Solid)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.83	3.94	<LOD	<LOD	<LOD	<LOD
FP-14-0806	NL	Tea and herbs for infusions (Solid)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.13	1.11	<LOD	<LOD	<LOD	<LOD
FP-14-0832	ES	Tea and herbs for infusions (Solid)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.63	6.98	<LOD	<LOD	<LOD	<LOD
FP-14-0879	EL	Tea and herbs for infusions (Solid)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0885	ES	Tea and herbs for infusions (Solid)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.96	2.50	<LOD	<LOD	<LOD	<LOD
FP-14-0886	ES	Tea and herbs for infusions (Solid)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.52	0.52	<LOD	<LOD	<LOD	<LOD
FP-14-0887	ES	Tea and herbs for infusions (Solid)	3.98	20.53	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	11.68	105.21	<LOD	<LOD	<LOD	<LOD
FP-14-0889	ES	Tea and herbs for infusions (Solid)	<LOD	5.81	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.82	2.84	<LOD	<LOD	<LOD	<LOD
FP-14-0890	ES	Tea and herbs for infusions (Solid)	0.16	0.30	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	6.80	15.65	<LOD	<LOD	<LOD	<LOD
FP-14-0891	ES	Tea and herbs for infusions (Solid)	0.15	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.21	0.77	<LOD	<LOD	<LOD	<LOD

Appendix J, cont'd. PA concentrations (µg/L in infusion) in positive (herbal) food supplement samples to be used as infusion. PA abbreviations are explained in Table 11

ID sample	Country	Type of supplement	Ly	LyNO	Mc	McNO	Re	ReNO	Sn	SnNO	Sp	SpNO	Sv	SvNO	Sk	Td
FP-14-0792	NL	Tea and herbs for infusions (Solid)	1.76	1.38	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0793	NL	Tea and herbs for infusions (Solid)	4.31	6.98	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0804	NL	Tea and herbs for infusions (Solid)	0.24	0.10	<LOD	<LOD	<LOD	<LOD	<LOD	0.13	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0805	NL	Tea and herbs for infusions (Solid)	0.81	1.41	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0806	NL	Tea and herbs for infusions (Solid)	0.88	1.29	<LOD	<LOD	5.15	<LOD	0.59	2.43	<LOD	<LOD	0.44	2.40	60.11	<LOD
FP-14-0832	ES	Tea and herbs for infusions (Solid)	3.49	9.52	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.32	<LOD
FP-14-0879	EL	Tea and herbs for infusions (Solid)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.59	0.43	<LOD	<LOD	0.38	0.84	18.19	<LOD
FP-14-0885	ES	Tea and herbs for infusions (Solid)	1.81	5.22	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.23	<LOD
FP-14-0886	ES	Tea and herbs for infusions (Solid)	0.53	0.60	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.22	<LOD
FP-14-0887	ES	Tea and herbs for infusions (Solid)	170.36	102.92	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0889	ES	Tea and herbs for infusions (Solid)	15.70	28.35	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0890	ES	Tea and herbs for infusions (Solid)	161.85	197.80	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
FP-14-0891	ES	Tea and herbs for infusions (Solid)	0.73	2.48	<LOD	<LOD	<LOD	<LOD	<LOD	0.18	<LOD	<LOD	0.12	0.32	<LOD	<LOD

ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
DE	Germany
EFSA	European Food Safety Authority
EL	Greece
Em	Echimidine
EmNO	Echimidine-*N*-oxide
Er	Erucifoline
ErNO	Erucifoline-*N*-oxide
ES	Spain
ESI	Electro Spray Ionisation
ESI+	Positive Electro Spray Ionisation
EU	European Union
Eu	Europine
EuNO	Europine-*N*-oxide
eV	electron volt
Fs	Florosenine
He	Heliotrine
HeNO	Heliotrine-*N*-oxide
Id	Indicine
IdNO	Indicine-*N*-oxide
Ir	Integerrimine
IrNO	Integerrimine-*N*-oxide
Im	Intermedine
ImNO	Intermedine-*N*-oxide
IS	Internal standard
ISO	International Organization for Standardization
IT	Italy
IUPAC	Union of Pure and Applied Chemistry
Jb	Jacobine
eJb	*epi*-Jacobine
JbNO	Jacobine-*N*-oxide
Jl	Jacoline
LB	Lower bound
Lc	Lasiocarpine
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
MS/MS	Tandem mass spectrometry
LcNO	Lasiocarpine-*N*-oxide
LOD	Limit of detection
LOQ	Limit of quantification
Ly	Lycopsamine
LyNO	Lycopsamine-*N*-oxide
MB	Middle bound
Mc	Monocrotaline
McNO	Monocrotaline-*N*-oxide
MMS	Matrix-matched standard
MMRS	Matrix-matched recovery standard
MOE	Margin of Exposure
MRM	Multiple reaction monitoring
m/z	mass over charge ratio
NL	the Netherlands

Ot	Otosenine
PA FB	Pyrrolizidine alkaloid free base
PANO	Pyrrolizidine alkaloid *N*-oxide
PAs	Pyrrolizidine alkaloids
QC	Quality control
Re	Retrorsine
ReNO	Retrorsine-*N*-oxide
Rd	Riddelliine
RdNO	Riddelliine-*N*-oxide
RSD	Relative Standard Deviation
RT	Retention time
S/N	Signal to noise (ratio)
Sn	Senecionine
SnNO	Senecionine-*N*-oxide
Sp	Seneciphylline
SPE	Solid-phase extraction
SpNO	Seneciphylline-*N*-oxide
Sv	Senecivernine
SvNO	Senecivernine-*N*-oxide
Sk	Senkirkine
Td	Trichodesmine
TdNO	Trichodesmine-*N*-oxide
UB	Upper bound
UHT	Ultra-high temperature processed
UK	United Kingdom
WHO	World Health Organization