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Title: Determination of Acrylamide in Dried Fruits and Edible Seeds Using QuEChERS extraction and LC Separation with MS Detection

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Keywords: Acrylamide; QuEChERS; LC-ESI-MS-Triple Quadrupole; Dried fruits; Dried prunes; Peanuts.

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Abstract: Acrylamide is a carcinogenic and neurotoxic process contaminant that is generated from food components during heat treatment, while it is absent in raw foodstuffs. Its level in food arouses great concern. A method for acrylamide extraction and determination in dried fruits (dried prunes and raisins) and edible seeds (almonds, hazelnuts, peanuts, pine nuts, pistachios, and walnuts) using a QuEChERS-LC-ESI-MS-Triple Quadrupole approach was set up. Linearity, sensitivity, accuracy, and precision of the method were satisfactory.
Dried prunes and peanuts were the only samples appreciably contaminated, 14.7-124.3 and 10.0-42.9 µg/kg, respectively, as a consequence of the drying process. In fact, prunes are dried at 70-80 °C for a quite long time (24-36 h), while peanuts undergo a roasting process at 160-180 °C for 25-30 min.
The relative standard deviations, accuracy, LOD, and LOQ show that the method provides a reliable approach to acrylamide determination in different matrices.

Ms. Ref. No.: FOODCHEM-D-15-03161R1

Dear Editor,

I am sending you a copy of the revised Ms. entitled: Determination of Acrylamide in Dried Fruits and Edible Seeds Using QuEChERS extraction and LC Separation with MS Detection.

All reviewers' comments were carefully considered and accepted in large part. English was revised, as well.

Yours sincerely,

Dr. Giuseppe Montevecchi

Ms. Ref. No.: FOODCHEM-D-15-03161R1

Reviewers' comments:

Reviewer #3

In my opinion, in the present form the manuscript titled 'Determination of Acrylamide in Dried Fruits and Edible Seeds Using QuEChERS extraction and LC Separation with MS Detection' described by Eleonora Laura De Paola, Giuseppe Montevecchi, Francesca Masino, Davide Garbini, Martino Barbanera, Andrea Antonelli can be recommended for publication in the Food Chemistry after minor revision.

My remarks and recommendations to the FOODCHEM-D-15-03161.R1 manuscript are as follows:

1. The matrix effect should be estimated as ratio of slopes (matrix/solvent).

QuEChERS sample preparation is a classical method to reduce matrix effect. Moreover, our study was carried out on different samples with very different matrices. For these reasons, we did not carry out a true "matrix effect study" but we considered the residual matrix effect (after QuEChERS purification) by spiking real sample technique.
For sake of precision and considering the reviewer observation, we rename 3.2.2 section "Accuracy evaluation", only.

2. Number of experiments used to estimate the SD?

Done in Materials and methods (lines 137-138) and in Table 6. Each sample extraction was carried out in triple.

3. Neither, there is in the article a comparison of the characteristics of the method with other previously published, for example a comparison of the mLOQs to other methods previously published. Please, indicate clearly the advantages of the method proposed compared to other methods previously published.

The general advantage are now presented in the Introduction (lines 90-93) and Conclusions (lines 259-262), while the comparison with other methods previously published are reported in 3.2.1. (lines 204-205) and Conclusions (line 261-262).

Determination of Acrylamide in Dried Fruits and Edible Seeds Using QuEChERS extraction and LC Separation with MS Detection

Eleonora Laura De Paola, Giuseppe Montevecchi, Francesca Masino, Davide Garbini, Martino Barbanera, Andrea Antonelli

Highlights: > Acrylamide, a toxic process contaminant generated during heat treatment of food > QuEChERS approach was for the first time optimized for dried fruits and edible seeds > 68 samples of dried fruits and edible seeds purchased on Italian market were tested > Method linearity, sensitivity, matrix effect, accuracy, and precision were evaluated > Dried prunes and peanuts were the only samples contaminated with acrylamide

Determination of Acrylamide in Dried Fruits and Edible Seeds Using QuEChERS extraction and LC Separation with MS Detection

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Abstract

Acrylamide is a carcinogenic and neurotoxic process contaminant that is generated from food components during heat treatment, while it is absent in raw foodstuffs. Its level in food arouses great concern. A method for acrylamide extraction and determination in dried fruits (dried prunes and raisins) and edible seeds (almonds, hazelnuts, peanuts, pine nuts, pistachios, and walnuts) using a QuEChERS-LC-ESI-MS-Triple Quadrupole approach was set up. Linearity, sensitivity, accuracy, and precision of the method were satisfactory.

Dried prunes and peanuts were the only samples appreciably contaminated, 14.7-124.3 and 10.0-42.9 μg/kg, respectively, as a consequence of the drying process. In fact, prunes are dried at 70-80 °C for a quite long time (24-36 h), while peanuts undergo a roasting process at 160-180 °C for 25-30 min.

The relative standard deviations, accuracy, LOD, and LOQ show that the method provides a reliable approach to acrylamide determination in different matrices.

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Keywords: Acrylamide; QuEChERS; LC-ESI-MS-Triple Quadrupole; Dried fruits; Dried prunes; Peanuts.

Chemical compounds studied in this article:

Acrylamide (PubChem CID: 6579)

1. Introduction

Acrylamide is a neo-formed contaminant (NFC), produced in food during manufacturing or home-cooking (Capuano & Fogliano, 2011). It is absent in raw foods and in raw materials used to make food, and it is produced and accumulated during thermal processing (FSA, 2012).

Global levels of dietary exposure to acrylamide indicate a human health concern (FAO/WHO, 2010).

Acrylamide was first classified as a potential carcinogen and neurotoxic to humans (Group 2A) based on its carcinogenicity in rodents in 1994 (IARC, 1994) and the suspicion was then endorsed in 2002 (WHO, 2002; SNFA, 2002; Tareke, Rydberg, Karlsson, Eriksson, & Tornqvist, 2002). For these reasons, acrylamide level has been strictly controlled by the authorities (EFSA, 2012) even if there is no existing legal limit for the concentration of this contaminant in foodstuffs. However, European Union fixed a maximum recommended level of 1000 $\mu g\ kg^{-1}$ for potato chips (EU, 2013).

Acrylamide is formed primarily in carbohydrate-rich foods treated at high temperatures (i.e. > 120 °C) (Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2000; Tareke et al., 2002). The predominant chemistry involves the Maillard reaction, that occurs by a condensation of the amino group of the asparagine and the carbonyl group of reducing sugars during heating (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Stadler et al., 2004; Zyzak et al., 2003). Browned crispy crusts in foods like French fries, potato chips, coffee, crackers, pretzel-like snacks, cereals, and browned breads have the highest levels of acrylamide (EPA, 2010).

In addition, other reaction routes are involved in acrylamide formation in food. Acrolein (propenal), an unsaturated aldehyde, can be an acrylamide precursor and it can be produced from triglycerides by strong heat treatment (Umano & Shibamoto, 1987). Acrylic acid (Yasuhara, Tanaka, Hengel, & Shibamoto, 2003) and wheat gluten (Claus, Weisz, Schieber, & Carle, 2006) are acrylamide precursor in other minor routes. Finally, 3-aminopropionamide is an effective precursor of

acrylamide in the absence of further catalysts, such as carbonyls (Granvogl, Jezussek, Koehler, & Schieberle, 2004; Granvogl & Schieberle, 2006; Granvogl & Schieberle, 2007).

Acrylamide has been detected in some food products that are processed at temperatures in the 98–116 °C range, and in high moisture conditions [i.e., canned black olives (not cured oil) and prune juice] (Roach, Andrzejewski, Gay, Nortrup, & Musser, 2003). It is clear that other pathways of formation below 120 °C can yield acrylamide, and these are being further evaluated (JIFSAN, 2004).

Factors that are particularly important for the Maillard reaction are the starting reactants (Yaylayan & Stadler, 2005), i.e. kind of sugar and amino acid (protein), temperature, time and water activity. The presence of metal salts (pro-oxidants), and inhibitors, such as antioxidants and sulfite, may have an impact.

During analysis, acrylamide, a small hydrophilic molecule, is usually extracted with water but a polar organic molecule, such as the more volatile acetonitrile, is a suitable alternative (Tateo & Bononi, 2003). To extract acrylamide, different authors used an aqueous solution with high-concentration of NaCl to inhibit the formation of emulsions (Young, Jenkins & Mallet, 2004), or water and 1-propanol on defatted samples (Biedermann, Biedermann-Brem, Noti, & Grob, 2002). Other authors introduced a deproteinating step (Gertz & Klostermann, 2002). However, all these sample manipulations can be bypassed by a QuEChERS approach (Mastovska & Lehotay, 2006). In comparison with a traditional strategy based on solid phase technique (SPE), the proposed method allows “one-pot” sample preparation thus limiting the amount of solvent used for the extraction. Remarkable time and money-per-sample savings are considerable, as well.

LC-MS/MS is the most used and authoritative method for acrylamide determination. Because its high sensitivity, LC-MS/MS avoids the derivatization step, that is time consuming and potentially unhealthy.

The aim of this study is to set up and apply the more efficient QuEChERS approach in order to extract and determine acrylamide in packed dried fruits (dried prunes and raisins) and some edible seeds (almonds, hazelnuts, peanuts, pine nuts, pistachios, walnuts).

2. Materials and methods

2.1. Sampling and grinding

Sixty-eight samples of packed dried fruits and edible seeds were purchased on the Italian market. In particular, dried prunes (13 samples) pitted (7 samples) and not pitted (6 samples), and raisins (7 samples) as dried fruits, and peeled almonds (2 samples), roasted and peeled hazelnuts (2 samples), roasted and salted pistachios (7 samples), pine nuts (7 samples) from Portugal (2 samples) and Italy (5 samples), walnuts (4 samples) with shell from USA (2 samples) and from Chile (2 samples), and roasted and salted peanuts (26 samples) with shell and from Israel (24 samples) and from Egypt (2 samples), as edible seeds.

A gross amount of 20 g of each sample was ground by Osterizer 12-speeds blender (Oster Manufacturing, Di Giovanni Srl, Bologna, Italy) for further elaboration.

2.2. Chemicals

All solvents and reagents were of analytical grade; acetonitrile (Chromasolv® Plus purity for LC-MS, Sigma-Aldrich®), petroleum ether, methanol (Chromasolv® Plus purity for LC-MS, Sigma-Aldrich®), and *n*-hexane were obtained from Fluka Sigma-Aldrich® S.r.l. (Milan, Italy). Anhydrous sodium sulphate was purchased from Carlo Erba Reagents S.p.A. (Rodano, Milan, Italy).

QuEChERS pouches containing $MgSO_4$ 4.0 g + NaCl 0.5 g were purchased from Agilent Technologies Italia S.p.A. (Milan, Italy). DisQuE QuEChERS tubes (1) containing $MgSO_4$ 8.0 g + CH_3COONa 2.0 g plus DisQuE QuEChERS tubes (2) containing PSA (Primary Secondary Amine – dSPE – technique) 25 mg and $MgSO_4$ 150 mg were purchased from Waters

S.p.A. (Milan, Italy). Acrylamide used as external standard was purchased by Sigma-Aldrich® S.r.l. (Milan, Italy) with a purity of 99 %.

2.3. QuEChERS protocol setup

Different protocols and materials for acrylamide extraction were tested. They included different clean-up steps (defatting by hexane or dispersive SPE clean-up), effect of injection solvent (acetonitrile), QuEChERS pouches (Agilent QuEChERS pouches containing $MgSO_4$ 4.0 g + NaCl 0.5 g or Waters QuEChERS tubes containing $MgSO_4$ 8.0 g + CH_3COONa 2.0 g), water volume (0, 2.5 mL, 5.0 mL), and sample weight (1.00 g, 2.50 g, 5.00 g).

2.3.1. Optimized extraction protocol for acrylamide

An aliquot of ground sample (2.50 g) was transferred into a 50-mL Falcon tube together with a ceramic homogenizer for QuEChERS. Then 5 mL of Milli-Q water and 10 mL of acetonitrile were added, and the tube was vigorously hand shaken for 1 min after the addition of each solvent. A prepared mix of QuEChERS pouch composed by $MgSO_4$ 4.0 g + NaCl 0.5 g was added and hand shaken for 1 min and for 3 min with a shaker (Unimax 2010, Heidolph Instruments Italia S.r.l., Milan, Italy) to separate acrylamide into the acetonitrile phase. The tubes were centrifuged for 3 min at 3000 rpm to separate the two layers. An aliquot of the upper layer (2 mL) was dried by a gentle nitrogen stream (20 min in a water bath at 40 °C). The sample was dissolved in 1 mL of Milli-Q water and filtered through a 0.22 μm PES membrane into a 2 mL vial that was loaded into autosampler chamber at controlled temperature, ready for analysis by LC-MS-MS. Each sample extraction was carried out in triple.

2.4. Acrylamide determination

The acrylamide determination was carried out by reverse phase liquid chromatography coupled with mass spectrometry system (Agilent Technologies, Waldbronn, Germany) consisting of a vacuum pump (Agilent 1200), a gas generator (API MM20 ZA; Peak Scientific Billerica, MA, USA), a degasser (Agilent 1200), a binary pump (Agilent 1200), an autosampler (Agilent 1200), a thermostated column compartment (Agilent 1200), and a mass spectrometer triple quadrupole (API 3200, AB Sciex Germany GmbH, Darmstadt, Germany). Samples (20 μL) were injected into a Gemini RP C_{18} column (Phenomenex, Torrance, CA, USA) (25 cm × 2 mm i.d. × 5 μm particle size × 110 Å pore size). The solvent system was 0.1 % formic acid in water (99.5 %, solvent A) and 0.1 % formic acid in methanol (0.5 %, solvent B) and the elution was carried out in isocratic mode (total run 7 min), with a flow rate of 0.25 mL/min at ambient temperature. The analysis was performed in double for all the samples.

The analyses were carried out in positive electrospray ionization mode (ESI+) and using the following conditions: curtain gas, 20.0 psi; collision activated dissociation, 7.0 (arbitrary units); ion spray, 5500.0 V; temperature, 700.0 °C; nebulizer gas, 70.0 psi; heater gas, 30.0 psi. The main MS parameters optimized for acrylamide determination were: declustering potential, 22.0 V; collision energy, 14.1 V; collision cell exit potential, 4.1 V; entrance potential, 6.0 V. The parent ion m/z was 72.1 and the qualifier ion m/z was 54.9. These conditions were optimized by injecting acrylamide solutions directly into the MS source.

Quantification was performed by external standard calibration and the chromatograms were acquired and processed by Analyst software version 1.5 (AB Sciex).

3. Results and discussion

3.1. Acrylamide extraction

3.1.1. Effect of defatting by hexane and dispersive SPE clean-up steps

Mastovska et al. (2006) used hexane to defat samples and dSPE to clean up the extract. However, in our samples the addition of 5 mL of hexane or the use of dSPE reduced acrylamide recovery at about 20 %, and 36 %, respectively. For these reasons, these two steps were not included in the protocol of acrylamide extraction.

3.1.2. Effect of injection solvent on acrylamide determination

Direct injection of the acetonitrile extract after QuEChERS altered the retention time during chromatography. In addition, acetonitrile is a poor solvent for lipids that precipitated trapping part of the acrylamide, thus lowering its recovery. To overcome this problem, two ways were carried out: (i) a pre-dilution of the acetonitrile (250 µL) with water (750 µL), (ii) the complete acetonitrile evaporation and the resolubilization of the sample with water.

Best results were achieved with the latter solution that was used throughout this study.

3.1.3. Effect of two different QuEChERS pouches

QuEChERS pouches with different composition ($MgSO_4$ 4.0 g + NaCl 0.5 g and $MgSO_4$ 8.0 g + CH_3COONa 2.0 g) were compared.

QuEChERS with NaCl gave the best recoveries (Table 1), probably as a consequence of the reduced emulsions (Young et al., 2004). On the contrary, QuEChERS with CH_3COONa had a negligible effect on recoveries despite the observations of Eriksson & Karlsson (2006).

3.1.4. Effect of water volume ($MgSO_4$-NaCl pouches)

To verify the effect of water volume on acrylamide recoveries, some tests on an acrylamide solution in acetonitrile (250-µg/kg) were carried out (Table 2). Different volumes of water were used: 0, 2.5, and 5.0 mL. To meet a reasonable compromise between recovery and a good sample dispersion, 5.0 mL were used for further experiments.

192 *3.1.5. Effect of sample weight*

193 Comparison of the recoveries of different sample weights was carried out (Table 3). A 2.50-g
194 sample amount was a reasonable compromise between the highest recoveries and the necessity to
195 have a sample amount large enough to be representative.

196

197 *3.2. Method evaluation*

198 *3.2.1. Linearity and sensitivity determination*

199 Increasing concentrations of acrylamide, from 1 μg/kg to 500 μg/kg, were used to study the range of
200 linearity. As nor EFSA neither FSA suggested limits for dried fruits, we used those ones
201 recommended for biscuits, wafers, and crisp bread (EFSA 2012; FSA 2012).

202 The coefficient of determination R^2 was 0.999, showing a very good linearity.

203 Sensitivity of the method was determined with the limit of detection (LOD, 2.0 μg/kg) and the limit
204 of quantification (LOQ, 5.0 μg/kg) that were calculated by the calibration curve. These values were
205 lower in comparison with what found in different studies carried out with SPE extraction (Wenzl,
206 Karasek, Rosen, Hellenäs, Crews, Castle & Anklam, 2006; Bortolomeazzi, Munari, Anese &
207 Verardo, 2012).

208

209 *3.2.2. Accuracy evaluation (recoveries) and inter-laboratory essay*

210 One sample for each matrix was spiked with 250 μg/kg of acrylamide. The recoveries were
211 calculated for each sample (Table 4). For calculations of real samples, correction factors based on
212 the recoveries for each matrix were applied. Calibration was verified each day with three spiked
213 samples at different concentrations.

214 To test the adaptability of the method to extract acrylamide from other matrices, it was used a
215 European reference matrix (ERM), crisp bread (ERM-BD272) used in Food Analysis Performance
216 Assessment Scheme (FAPAS) for proficiency test 3024 (FAPAS, 2009; Koch, Bremser, Koeppen,
217 Siegel, Toepfer, & Nehls, 2009) to evaluate inter-laboratory variability. Even applying the poorest

recovery (61 %), the concentration of acrylamide was in the range of acceptability (Table 5). The same test was then applied to rusks, as this matrix was the most similar to ERM (Koch et al., 2009). Rusks were added with 250 μg/kg acrylamide and, even in this case, results were in the range of acceptability (Table 5).

3.2.3. Evaluation of precision: repeatability

For the determination of the precision, four levels of contamination were considered: (i) a sample not contaminated (pine nuts, acrylamide < LOD), (ii) a sample with a not quantifiable concentration of acrylamide (pistachios, acrylamide < LOQ), (iii) a sample with a medium amount of acrylamide (peanuts, 42.86 μg/kg), and (iv) a sample with a high concentration of acrylamide (dried prunes, 124.26 μg/kg). Each sample was extracted and injected three times. With acrylamide lower concentrations (5 - 25 μg/kg) relative standard deviation (RSD) was 20 %, while with higher acrylamide concentrations (26 - 124 μg/kg) RSD was 10 %.

3.3. Results obtained on real samples

Raisins, almonds, hazelnuts, pine nuts, and walnuts showed values lower than LOD, as well as pistachios, except for one sample that was lower than LOQ. For this reason the results of these samples are not shown.

These very low concentrations were expected because dried fruits and edible seeds are generally not subjected to high temperature treatments. In literature, it was reported that acrylamide levels in almonds were below 200 μg/kg if the roasting temperature was below 146 °C (Zhang, Huang, Xiao, Seiber, & Mitchell, 2011). These data were recently confirmed by Schlörmann and coll. (2015).

Dried prunes and peanuts (Table 6) showed acrylamide content ranged from 14.74 μg/kg to 124.26 μg/kg, and from 6.16 μg/kg to 42.86 μg/kg (with two samples < LOQ and one < LOD), respectively. The RSD were within 20 %, very good results if compared with 47 % found for roasted kernel (Amrein, Lukac, Andres, Perren, Escher, & Amadò, 2005) and 60 % for dried prunes

(Amrein, Andres, Escher, & Amadò, 2007). The lower manipulation of the sample was likely the reason of these good results.

Amrein and coll. (2007) detected from 730 to 1680 μg/kg of acrylamide in dried prunes. They also heated again dried prunes at 120 °C for 40 min and they found an increase in acrylamide content (up to 2240 μg/kg). In this case, time is the key factor for acrylamide formation. In fact, even if the drying process of the prunes is carried out at quite low temperature (70-80 °C), the time of exposure is long enough (24-36 h) to allow acrylamide formation. Moreover, the drying process (up to about 18 % moisture) enhances sugar and asparagine concentration (Amrein et al., 2007), thus fostering acrylamide formation.

In the case of peanuts, the roasting temperature is the key factor for acrylamide accumulation. In fact, temperatures are considerably higher (160-180 °C) for 25-30 min of roasting time.

4. Conclusions

The study describes a quick and easy method for acrylamide determination by QuEChERS approach coupled to LC-ESI-MS-Triple Quadrupole technique with a "one-pot" sample preparation. The method was applied for the first time to food matrices such as dried fruit (prunes and raisins) and some edible seeds. Besides solvent saving, the proposed approach is more sensitive and repeatable, with lower LOD and LOQ.

Results confirm that acrylamide was found with significant concentrations in dried prunes and peanuts as a consequence of the thermal process they were submitted. In general, data agree with those of literature. Other products have shown minimal acrylamide amounts, thus posing reduced harm to humans.

For these reasons and for its flexibility to different products, this method is very promising for acrylamide determination in other matrices, as well.

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Table 1.

Comparisons between two different QuEChERS compositions.

QuEChERS pouches	Sample weight (g)	Recoveries (%)	
		Almonds	Pistachios
$MgSO_4$ - NaCl	1.00	60 ± 5	69 ± 6
	2.50	70 ± 2	73 ± 2
$MgSO_4$ - CH_3COONa	1.00	54 ± 7	30 ± 8
	2.50	58 ± 7	58 ± 6

Table 2.

Composition of the trials and recoveries (%) found using different water contents to disperse the sample and dissolve acrylamide. The volume of acetonitrile was 10 mL and the final acrylamide concentration was 250 μg/kg.

Trials	H_2O volume (mL)	$MgSO_4$-NaCl	Recoveries (%)
1	No	No	40 ± 8
2	No	Yes	83 ± 6
3	2.5 mL	Yes	107 ± 3
4	5.0 mL	Yes	117 ± 4

Table 3.

Effect of sample weight on recoveries (%) of acrylamide (250-µg/kg spike).

Sample weight (g)	Dried Prunes	Raisins	Almonds	Pistachios
1.00	84 ± 10	72 ± 8	70 ± 9	68 ± 8
2.50	61 ± 2	61 ± 3	81 ± 8	73 ± 7
5.00	36 ± 7	34 ± 8	54 ± 9	38 ± 8

Table 4.

Recoveries (%) calculated for each matrix, spiked with 250 μg/kg of acrylamide.

Samples	Recoveries (%)
Almonds	70 ± 4
Hazelnuts	76 ± 3
Peanuts	77 ± 3
Pine nuts	63 ± 8
Pistachios	62 ± 7
Walnuts	82 ± 6
Dried prunes	61 ± 2
Raisins	61 ± 3

Table 5.

FAPAS proficiency test results. [a]The range of acceptability expected by FAPAS included values in the range of Z score between -2 and 2. It was calculated that Z = -2 corresponded to 165 μg/kg of acrylamide, while Z = 2 to 377 μg/kg, so that Z = 0 is 271 μg/kg. The standard deviation to apply (± 106) was corresponding to 39% RSD (FAPAS, 2009).

Sample	Recoveries (%) applied	Acrylamide content (μg/kg)	Range of acceptability[a] (μg/kg) ± standard deviation
Crisp bread (ERM-BD272)	61	167	271 ± 106
Rusks (spiked with 250 μg/kg of acrylamide)	61	277	271 ± 106

Table 6.

Acrylamide content (µg/kg) in dried prunes (DP) and peanuts (PE). Each sample was triplicated. LOD = limit of detection; LOQ = limit of quantification; SD = standard deviation.

	DP1	DP2	DP3	DP4	DP5	DP6	DP7	DP8	DP9	DP10	DP11	DP12	DP13
µg/kg	14.75	71.15	76.07	71.80	72.46	45.25	124.26	44.59	14.92	101.31	104.59	34.75	16.75
SD (±)	2.95	7.11	7.61	7.18	7.25	4.52	12.43	4.46	2.98	10.13	10.46	3.48	3.35
	PE1	PE2	PE3	PE4	PE5	PE6	PE7	PE8	PE9	PE10	PE11	PE12	PE13
µg/kg	11.22	26.49	<LOQ	29.87	18.65	21.71	<LOQ	9.71	9.74	14.78	12.88	21.84	16.13
SD (±)	2.24	5.30		2.99	3.73	4.34		1.94	1.95	2.96	2.58	4.37	3.23
	PE14	PE15	PE16	PE17	PE18	PE19	PE20	PE21	PE22	PE23	PE24	PE25	PE26
µg/kg	13.04	<LOD	9.38	12.29	14.83	10.49	6.16	7.45	15.40	1.08	3.30	32.73	42.86
SD (±)	2.61		1.88	2.46	2.97	2.10	1.23	1.49	3.08	0.22	0.66	3.27	4.29