Research Article

Mioara Negoită, Adriana Laura Mihai*, Gabriela Andreea Horneț, Denisa Eglantina Duță

Development of SPE clean-up procedure for acrylamide determination from potato-based products by GC-MS/MS

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Abstract: The aim of this work was to develop an analytical method for determining acrylamide in potato-based products by gas chromatography and tandem mass spectrometry analysis (GC-MS/MS) using solid-phase extraction (SPE) clean-up. Different conditions for extraction and clean-up of AA extracts were studied on a potato crisps reference material (RM) with the value of $625 \pm 45 \mu\text{g/kg}$: the solvent volume used for AA elution, the extraction water temperature, the volume of hexane used, and also the addition of Carrez solutions. The SPE cartridge sorbents contain silica-based C-18 groups (anion and cation exchangers) and polymers (polystyrene-divinylbenzene). After SPE clean-up, extracts were derivatized with bromine compounds. A good efficiency for AA extraction and a cleaned-up extract from this matrix were obtained when the SPE procedure was carried out with water at room temperature, simultaneous with hexane, without Carrez solutions, by using two types of SPE cartridges (Isolute Multimode; Isolute ENV⁺), and 5 mL of elution solvent. The SPE clean-up procedure functionality was demonstrated by the results obtained in the Food Analysis Performance Assessment Scheme proficiency test (z-score: -0.8) on French fries (precooked) matrix and also by comparative analysis with a laboratory procedure, validated and accredited on cereal-based food matrices, in which liquid–liquid extraction and clean-up through a florisil column were realized ($\text{RSD}_{(R)} = 2.23\text{--}5.10\%$).

Keywords: acrylamide, florisil, derivatization, potato crisps, French fries

1 Introduction

Acrylamide (AA) is a process chemical contaminant obtained when food is subjected to temperatures higher than 120°C and low moisture. Since 2002 when acrylamide was found in food, many organizations studied its effects being classified as a “probable human carcinogen” by the International Agency for Research on Cancer (IARC) (1994), as “reasonably anticipated to be a human carcinogen” by the US National Toxicology Program (NTP) (2005), and as “likely to be carcinogenic to humans” by the US Environmental Protection Agency (EPA) (2010). Also, the European Food Safety Authority (EFSA) confirmed that AA presence in food is a public health concern (2015).

This contaminant is generated during cooking at high temperatures, in certain types of starchy foods, as a consequence of the Maillard browning reaction, in which the free amino acid asparagine and reducing sugars or other carbonyl compounds interact. AA is formed predominantly in baked or fried foods rich in carbohydrates, mainly derived from plant sources, including potatoes and cereals. Potato-based products are one of the main sources of AA dietary intake, together with coffee and cereal-based products. Statistical data revealed that the consumption of potato chips in Romania increased in the last years, being a popular product, eaten at least once a week by 31% of Romanians living in urban areas (FRD Center 2016). In a toolbox proposed by FoodDrinkEurope (2019), potato-based products have been categorized as potato-based snacks versus French fries and other cut (deep-fried) potato products. The benchmark level for AA in French fries was set to $500 \mu\text{g/kg}$, while for the potato-based snacks it was set to $750 \mu\text{g/kg}$ by the European Commission

* **Corresponding author: Adriana Laura Mihai**, Chromatography Department, National Research & Development Institute for Food Bioresources, IBA Bucharest, 6 Dinu Vintilă Street, District 2, Bucharest, 021102, Romania, e-mail: mihai_laura21@yahoo.com
Mioara Negoită, Gabriela Andreea Horneț: Chromatography Department, National Research & Development Institute for Food Bioresources, IBA Bucharest, 6 Dinu Vintilă Street, District 2, Bucharest, 021102, Romania

Denisa Eglantina Duță: Scientific Department, National Research & Development Institute for Food Bioresources, IBA Bucharest, 6 Dinu Vintilă Street, District 2, Bucharest, 021102, Romania

(2017). The AA level of potato-based products is influenced by the content of asparagine and reducing sugars of potato cultivar, the storage and transport conditions, frying temperature and time, moisture, and oil uptake. Yang *et al.* (2016) showed that the AA content of French fries is influenced by the potato cultivars and frying temperature, the AA level being affected by the asparagine, reducing sugars, and sucrose content of fresh potatoes, the oil uptake and moisture content of potato strips.

When a large number of food samples need to be investigated, a rapid analysis of AA in food matrices has become increasingly important. Therefore, the processing of food samples includes a series of steps, such as homogenization, AA extraction, fat removal, protein precipitation, purification and concentration of extracts, derivatization, and chromatographic conditions, which should be optimized to minimize their duration. In the processing stages, the analyte is transferred from the matrix into an extract as free as possible of interference compounds and compatible with the chromatographic system and the detection method. Determining AA in food is a difficult process because of the diversity of food matrices, especially the AA properties. AA presents good solubility in polar solvents such as water and methanol, in contrary with nonpolar solvents such as hexane, where the solubility is low. The high solubility, the low molecular weight, and volatility of AA determine the extraction strategy and analytical technique for AA analysis (Nematollahi *et al.* 2019).

Sample preparation is an important step in AA analysis and various extraction and purification procedures, such as: liquid extraction (Fernandes and Soares 2007), microextraction (Nematollahi *et al.* 2019), dispersive liquid-liquid microextraction (Galuch *et al.* 2019), matrix solid-phase dispersion (Fernandes and Soares 2007), solid-phase extraction (SPE) (Sanny *et al.* 2012; Dias *et al.* 2017; Lambert *et al.* 2018; Genovese *et al.* 2019), and headspace solid-phase microextraction (HS-SPME) (Ghiasvand and Hajipour 2016), were studied.

Over the years, many analytical methods were developed for the AA determination. The most used methods for AA analysis are based on liquid or gas chromatography, both coupled with mass spectrometry (LC-MS/MS) (Sanny *et al.* 2012; Lambert *et al.* 2018) or gas chromatography and tandem mass spectrometry analysis (GC-MS/MS) (Dias *et al.* 2017)) after AA extraction and purification. In order to be analyzed at GC-MS, AA derivatization is required for improving the extraction capability, increasing the sensitivity and selectivity, and improving the precision of assays (Liu *et al.* 2008). Taking this into account, it can be considered that this technique effectively compensates for a difficult and

time-consuming derivatization process for AA determination (Hoenicke *et al.* 2004; Cheng *et al.* 2006). The most popular method for AA derivatization involves bromination before the analysis (Zhang *et al.* 2005). In the derivatization process, bromine compounds are used and AA is transformed into 2,3-dibromopropanamide (2,3-DBPA).

The aim of the present study was to develop a procedure for extraction and clean-up of AA extracts, in order to determine the AA content of potato-based products. Results were compared with the data obtained by using another laboratory procedure, validated and accredited on cereal-based food matrices, in which clean-up through a florisil column (FC) was realized. The purpose of the developed method is to eliminate some sample preparation steps, to use less volumes of solvents, and to be safer for the analyst. In order to optimize the SPE procedure and obtain a maximum efficiency and a cleaned-up extract, several factors were investigated, such as: the temperature of water used for extraction, addition of hexane and Carrez solutions, and the solvent volume used for AA elution.

2 Materials and methods

2.1 Food matrices

In this study, a potato crisps reference material (TET043RM) with a reference value of AA, $625 \pm 45 \mu\text{g/kg}$ was used. Also, commercial samples of chips, French fries purchased from local supermarkets, and fast foods from Bucharest, Romania were analyzed. All samples were finely ground and homogenized in a laboratory mixer (Büchi B-400, BÜCHI Labortechnik AG, Switzerland) and then stored in the freezer at -21°C . In order to demonstrate the functionality of the SPE clean-up procedure, two proficiency tests (PTs) launched by the Food Analysis Performance Assessment Scheme (FAPAS) program, PT 3095 (French Fries – precooked) and PT 3094 (biscuit – cookie), were performed, processing the two food matrices, in parallel, through two procedures, SPE and the existing procedure developed in the laboratory, through cleaning up with a FC.

2.2 Reagents and reference standards

AA of concentration $1,004 \mu\text{g/mL}$ in methanol (99% purity) was purchased from Restek (Benner Circle, Bellefonte, US). The internal standard (IS) of labeled AA ($1,2,3\text{-}^{13}\text{C}$ labeled AA) (99% purity) of concentration $1,000 \mu\text{g/mL}$ in methanol

(+100 ppm hydroquinone) was acquired from Cambridge Isotope Laboratories (Andover, MA, USA).

For SPE experiments, two SPE cartridges were used: SPE-1 – an Isolute Multimode cartridge (1,000 mg/6 mL, Biotage AB, Uppsala, Sweden) and SPE-2 – an Isolute ENV⁺ cartridge (500 mg/6 mL, Biotage AB, Uppsala, Sweden). The SPE-1 cartridge contains silica-based C-18 groups as well as anion and cation exchangers; AA is not retained in the column, but many matrix components (non-polar and ionic compounds) that could exert interference are retained. At the second SPE step, the SPE-2 contains a polymer-based phase with high capacity to bind AA. SPE-2 is an especially dedicated SPE cartridge to retain highly polar compounds. The elution solvent for AA extraction is 60% methanol in water. Potassium bromide, acetic acid (glacial) 100%, potassium hexacyanoferrate(II) trihydrate, and zinc sulfate heptahydrate were all obtained from Merck (Darmstadt, Germany) and were of analytical grade. Hydrobromic acid (ACS reagent, 48%) was from Alfa Aesar (Thermo Fisher Scientific, USA) and bromine 99.6% for analysis was from Acros Organics (Israel). Sodium sulfate anhydrous AR, granular, florisil PR 60/100 mesh, and all solvents (ethyl acetate, acetone, *n*-hexane, and methanol) picograde for residue analysis were from LGC Promochem GmbH (Wesel, Germany). Ultrapure water (resistivity of 0.055 $\mu\text{S}/\text{cm}$) was obtained through a PURELAB Option-S7 and PURELAB Ultra Ionic system (Elga Labwater, High Wycombe, UK).

2.3 Standard solutions

Stock solutions of AA and IS of concentration 100 mg/L were prepared from the standard solutions in amber vials by dissolving in ultrapure water. Working solutions of AA of concentrations 10, 1, and 0.1 mg/L and working solution of IS (1 mg/L) were prepared by diluting the stock solution with ultrapure water. Before extraction, to each sample a volume of 440 μL of the working IS solution was added. All stock and working solutions were stored in a refrigerator at 4°C.

The calibration solutions were prepared, according to the procedure developed previously in the laboratory (Negoiță and Culețu 2016), without performing the purification step. The final residue was redissolved in 1,000 μL ethyl acetate and 100 μL triethylamine. Aliquots from working solutions of AA were diluted with water to give calibration solutions of 50, 100, 200, 300, 400, 500, 750, 1,000, 2,000, 3,000, 4,000, and 5,000 $\mu\text{g}/\text{L}$ concentration, all containing 400 $\mu\text{g}/\text{L}$ of IS.

2.4 Equipment

GC-MS/MS analyses were performed with a gas chromatograph (TRACE GC ULTRA) coupled with triple quadrupole mass spectrometer (TSQ Quantum XLS), both from Thermo Fisher Scientific (USA). The analytical separation was performed in a capillary column TraceGOLDTM TG-WaxMS (30 m $\times$ 0.25 mm I.D.; 0.25 μm) from Thermo Fisher Scientific (USA). Sample insertion was performed with an automatic injector (Right PTV) using TriPlus AS autosampler (Thermo Fisher Scientific, USA). The SPE extractions were performed in a vacuum manifold (HyperSep Universal Vacuum SPE Manifold) from Thermo Fisher Scientific (USA) with capacity for 16 columns. Solvent evaporation was performed in a Büchi Rotavapor (model R-210, Büchi Labortechnik AG, Germany). The evaporation, under a stream of nitrogen, was carried out with a nitrogen generator (Domnick Hunter, USA).

2.5 GC-MS operating conditions

2.5.1 Gas chromatography

The carrier gas was helium (constant flow rate at 1.6 mL/min). The sample injection volume was 1 μL , which was injected into a PTV injector, in a split mode at a split ratio of 1:10. The injector temperature was set to 220°C. The oven temperature was set to 65°C and maintained for 1 min, then increased on ramp 1 with 15°C/min up to 170°C, on ramp 2 with 5°C/min up to 200°C, and finally on ramp 3 with 40°C/min up to 240°C, maintaining at this temperature for 15 min (total run time is 30 min).

2.5.2 Mass spectrometry

The analyses were performed in the electron impact ionization operation mode (EI⁺ mode – 70 eV), with the acquisition mode “selected reaction monitoring – SRM” and ion scanning mode “product”. AA and the IS were identified by the ion fragments corresponding to the derivatized ions, 2-BPA (2-bromopropenamide) and 2-BP (¹³C₃)A. The precursor ions with m/z 151 and 154 fragmentation were achieved with argon (1 mTorr), leading to the formation of product ions (daughter) with m/z 70 (2-BPA) and 73 (2-BP(¹³C₃)A), being used for quantification. The AA concentration calculation in the test samples was based on the ratio of the peak area

corresponding to the product ions, with m/z 70 and 73 for 2-BPA and 2-BP($^{12}\text{C}_3$)A, respectively.

2.6 Sample preparation

The procedure for sample preparation of potato-based products was achieved by improving the existing developed and validated method from laboratory with florisil clean-up (FC) (Negoită and Culețu 2016) in terms of AA extraction and clean-up.

2.6.1 The FC procedure

The method previously developed in the laboratory (FC) involves liquid–liquid extraction and purification with Carrez solutions and on a chromatographic column (300 mm $\times$ 11 mm I.D.) containing calcinated sodium sulfate and activated florisil. The first extraction step was performed with 30 mL hot water (60°C), added to 1.5 g sample (ratio sample:water = 1:20), followed by a purification step with Carrez I and II solutions (440 μL each) for protein and carbohydrate removal, and then by a centrifugation step (30 min at $6,000 \times g$ at 5°C) in a centrifuge with cooling (5804R, Eppendorf, Germany). The obtained supernatant was derivatized with 7.5 g KBr, 20–100 μL HBr, and 10 mL saturated bromine–water solution (around 1.6%) and let on a shaking water bath below 4°C, for at least 2 h. After the end of the derivatization reaction, bromine in excess was removed by adding around 1,000 mL of 1 mol/L sodium thiosulfate, until the yellow color disappeared. The derivatized compound (2,3-DBPA) was extracted with 70 mL mixture of ethyl acetate:hexane (4:1, v/v). The AA derivatized extract was concentrated in two steps. In the first step, a vacuum evaporation system was used and the extract was concentrated to ~ 2 mL. In the second step, the extract was evaporated to dryness under a nitrogen stream. The residue dissolved in 50 mL hexane was purified on a chromatographic column filled with activated florisil and calcinated sodium sulfate, previously conditioned with 20 mL hexane. The 2,3-DBPA derivative was eluted with 150 mL acetone, then concentrated until dryness and the residue was redissolved in 400 μL ethyl acetate and 40 μL triethylamine. The final extract (2-BPA) was filtered through a 0.2 μm regenerated cellulose microfilter (17 mm diameter, Spartan 13RC, Whatman GmbH, Dassel, Germany) directly in a vial and analyzed by GC-MS/MS in SRM mode.

The validation parameters for the FC procedure used for AA quantification from bread, biscuits, and other bakery products were described by Negoită and Culețu (2016), and the method was characterized by good sensitivity, with a limit of detection (LOD) of 1.23 $\mu\text{g/kg}$ (0.008 mg/L) and a limit of quantification (LOQ) of 3.7 $\mu\text{g/kg}$ (0.025 mg/L), respectively. The relative standard deviations (RSD) for repeatability and reproducibility were 0.4–4.9% and 2.23–5.10%, respectively. The recovery was in the range of 97%–105%.

2.6.2 The SPE procedure

The SPE clean-up procedure involves the simultaneous achievement of these stages on two SPE cartridges. Experimental variants (Table 1) were carried out to establish the optimum conditions for extraction and purification by using SPE cartridges in order to obtain a maximum efficiency of the analyte extraction from the food matrix and to obtain a sufficiently cleaned-up extract for chromatographic analysis. Thus, samples were weighed (0.5 g) in centrifuge vials of 50 mL and were spiked with 440 μL IS (concentration of 1 mg/L), while water at 60°C and at room temperature (ratio 1:20) and hexane (5–10 mL) were added and samples were homogenized for 60 min in a vortex mixer. Next, the samples were centrifuged for 20 min at $6,000 \times g$ at 5°C. In some variants, the aqueous phase separated was treated with Carrez solutions (I and II, 150 μL each) and after that, it was centrifuged. The supernatant (10 mL) obtained after centrifugation was passed through the SPE cartridges. Conditioning and washing of SPE 1 and SPE 2 cartridges were carried out, according to SR EN 16618:2015 (28). In order to establish the optimum elution conditions for AA extraction, different volumes (1–10 mL) of methanol:water mixture (60:40, v/v) were used. Elution and residual solvents were collected and the extract obtained was derivatized with 1 g KBr, 20 μL HBr, and 1 mL saturated bromine–water solution (around 1.6%). Derivatization took place on a shaking water bath below 4°C, for at least 1 h and at the end of the derivatization reaction, bromine in excess was removed by adding around 100 mL of 1 mol/L sodium thiosulfate. After derivatization, the extract was concentrated until dryness and the residue was redissolved in 400 μL ethyl acetate and 40 μL triethylamine. The final extract (2-BPA) was filtered through a 0.2 μm regenerated cellulose microfilter directly in a vial and analyzed by GC-MS/MS in SRM mode. All experiments were reported as the mean of minimum three replicate measurements.

Table 1: Experimental variants with the working conditions of SPE clean-up procedure

Variants	Phase elution/elution volume (mL)			Water temperature, °C		Hexane, mL		Carrez, 150 µL each	
	One phase	Two phases		Room	60	5	10	I	II
V1	10	—	—	—	X	—	X	X	X
V2	—	5	5	—	X	—	X	X	X
V3	5	—	—	—	X	—	X	X	X
V4	—	4	1	—	X	—	X	X	X
V5	—	3	2	—	X	—	X	X	X
V6	—	2	3	—	X	—	X	X	X
V7	5	—	—	X	—	—	X	X	X
V8	5	—	—	X	—	X	—	X	X
V9	5	—	—	X	—	—	X	—	—

A comparison of the two sample preparation procedures is presented in Figure 1.

2.7 Establishing the optimal SPE conditions for AA extraction from potato-based products

The optimal conditions of the developed SPE procedure were established following the performance and evaluation of nine experimental variants (Table 1), by varying the following factors: solvent volume used for AA elution (elution with 5 or 10 mL in a single- or two-phase elution); temperature of extraction water (room temperature or 60°C); the volume of hexane used for defatting the samples (5 or 10 mL); and addition of Carrez solutions.

In the case of experimental variants V1–V6, water at 60°C, Carrez solutions, and 10 mL of hexane were added for defatting the samples and the volumes of solvent used at AA elution through SPE-2 cartridge were varied. These variants were performed and evaluated in terms of achieving a maximum efficiency of analyte extraction from the food matrix and obtaining clean chromatograms, without interferences.

The aim of V3 and V7 (variation of water temperature), V7 and V8 (variation of hexane volume used for defatting the samples), and V7 and V9 (with or without Carrez solutions) variants was to reduce some additional steps from the FC procedure.

For evaluation of the optimal working conditions, the following acceptability criteria were required:

- In the case of V1–V6: selection of the variant (s), in which clean chromatograms with the highest S/N (signal/noise ratio) and areas of the AA peak are obtained.
- In the case of V7–V9:

- (a) The difference (dif., %) between the mean concentration value of the RM determined in the experimental variant (C_V) and the certified concentration value of the RM (C_{RM}) shall be less than or equal to $\pm 10\%$:

$$\text{Dif. (\%)} = ((C_V - C_{RM}) \times 100) / C_{RM}$$

- (b) The absolute difference (Δ_m) between C_V and C_{RM} shall be $\leq$ with the expanded uncertainty (U_Δ) (Linsinger 2010):

$$\Delta_m \leq U_\Delta \text{ where, } \Delta_m = |C_V - C_{RM}|; U_\Delta = k \times u_\Delta$$

U_Δ – the expanded uncertainty of the results obtained by multiplying the combined uncertainty (u_Δ), with the coverage factor (k), corresponding to a confidence level of approximately 95%

$$(k = 2)(U_\Delta = 2 \times u_\Delta)$$

$$u_\Delta = \sqrt{(u_V)^2 + (u_{RM})^2}$$

u_V – the combined uncertainty of the measurement result from the experimental variant was considered as the standard deviation (SD) of the number of measurements (n); $u_V = \text{SD} / \sqrt{n}$.

$$U_{RM} - \text{uncertainty of } C_{RM} \text{ value } C_{RM} = u_{RM} / k;$$

$$u_{RM} = 45/2 \mu\text{g/kg} = 22.5 \mu\text{g/kg}.$$

Based on the optimal variant established, some analytical parameters of the developed procedure were evaluated using the RM with the value of $625 \pm 45 \mu\text{g/kg}$: linearity, repeatability, interlaboratory reproducibility, sensitivity, and recovery. Linearity was determined by using two calibration curves in the ranges of 50–500 $\mu\text{g/L}$ and 400–5,000 $\mu\text{g/L}$. For repeatability, six replicates of the RM were realized and this parameter was evaluated by the RSD (%). The reproducibility was evaluated by participation in the interlaboratory proficiency testing (PT 3095) organized in 2019 by the FAPAS program on

Figure 1: Diagram of the sample preparation steps based on the FC procedure and optimal conditions for SPE procedure.

French fries (precooked). The method's sensitivity was evaluated by LOD and LOQ, which were determined by serial dilutions of the RM. In order to determine the recovery, a standard solution of AA with the concentration of 1 mg/L was used for sample spiking at different concentration levels (0.5, 1, and 1.5% of the benchmark level of 750 µg/kg).

3 Results and discussions

3.1 Optimization of SPE procedure

To achieve the best extraction and quantification conditions for AA analyses, several factors, such as the volume of solvent used for AA elution, the temperature of extraction water, the volume of hexane used for defatting the sample, and the chemical purification of

the AA extracts, were investigated. Purification of AA extracts by the SPE procedure, before chromatographic analysis, eliminates several interfering compounds and increases the method's accuracy.

3.1.1 Influence of solvent volume used for AA elution

In order to determine the optimum volume for achieving a maximum elution of the AA extraction from the food matrix, the experimental variants presented in Table 1 (V1–V6) were realized. The extracts obtained by passing the solvent (in one- or two phases) through the SPE-2 cartridges (Isolute ENV⁺) were collected separately in vials and subjected to derivatization, concentration, and injection at GC.

From the results obtained (Table 2), it is observed that the maximum efficiency of AA extraction was achieved when the elution was carried out with a volume of 5 mL solvent. In V2, where a two-phase

Table 2: AA content of RM (potato crisps, $625 \pm 45 \mu\text{g/kg}$) obtained by using different volumes of elution solvent

Volumes of elution solvent (variants)	Phase elution	Elution volume (mL)	Results	
			Concentration, $\mu\text{g/kg}$	S/N, A_{AA}
10 mL (V1)	One phase	10 mL	627.02	S/N = 13.727; A_{AA} = 539.404
10 mL (V2)	2 phases	5 mL	667.46	S/N = 42.906; A_{AA} = 3.439.955
		5 mL	<LOQ	S/N = 613; A_{AA} = 21.129
5 mL (V3)	One phase	5 mL	639.02	S/N = 42.816; A_{AA} = 3.067.738
5 mL (V4)	2 phases	4 mL	680.33	S/N = 36.907; A_{AA} = 3.493.972
		1 mL	<LOQ	S/N = 1.412; A_{AA} = 29.201
5 mL (V5)	2 phases	3 mL	786.71	S/N = 40.466; A_{AA} = 3.485.866
		2 mL	800.30	S/N = 2.075; A_{AA} = 79.071
5 mL (V6)	2 phases	2 mL	631.64	S/N = 31.463; A_{AA} = 2.693.111
		3 mL	660.52	S/N = 11.478; A_{AA} = 569.873

LOQ – limit of quantification ($20.83 \mu\text{g/kg}$), S/N – signal/noise ratio, A_{AA} – acrylamide peak area.

elution was carried out (5 mL + 5 mL), it was observed that after the first elution volume, the signal/noise ratio (S/N) and the AA peak area (A_{AA}) were the largest, and after the second elution volume addition, S/N and A_{AA} were smaller than those of the procedural blank realized with the same procedure. This denotes that AA was completely eluted with the first volume of 5 mL and the volume added in the second phase is in excess and inefficient. Thus, the volume of 10 mL of solvent in a single elution phase (V1) is too much for AA elution, as with this volume other compounds from the sorbent of SPE-2 cartridge are eluted, which give interferences and reduce S/N by about three times and A_{AA} by about 5–6 times, compared to the first elution volume of 5 mL in V2. The volume of 5 mL for the first elution phase in V2 is confirmed by the results of the tests performed under V3 conditions. In V3 variant, AA elution was performed with a 5 mL volume in a single phase, sufficient for AA elution, the highest values of the A_{AA} and S/N being obtained.

In order to acquire higher elution levels of AA, compared to the use of a volume of 5 mL solvent, in a single-phase elution, smaller volumes of elution (≤ 5 mL) (V4, V5, and V6) were also tested. Thus, in V4, the AA elution with a volume of 5 mL, in two elution phases determined for the first elution a S/N (36.907) close to that obtained when using 5 mL solvent (first elution V2 and elution from V3).

A small S/N (1.412) and A_{AA} (29.201) were determined for V4 when 1 mL was used for the second elution. In V5 and V6, it is observed that AA is eluted both in the first and in the second elution volumes. Thus, the volume of 5 mL of elution solvent used in a single phase

is optimal for an efficient AA extraction through the SPE-2 cartridge.

3.1.2 Influence of water temperature on AA extraction

For AA extraction from the RM by applying the SPE procedure, tests were performed, where the water temperature used for extraction was: 60°C (V3) and room temperature (V7) was the variable factor (Table 1). AA is highly soluble in water, minimizing the dissolution of hydrophobic compounds in food, as well as co-extraction with other undesirable compounds, such as salts, proteins, and carbohydrates, which could interfere with AA, if not removed by purification. The results obtained (Table 3) for the tests carried out in the two variants were satisfactory, fulfilling the required conditions imposed for (a) and (b).

Since there are no significant differences between the C_V determined under V3 and V7 conditions and the C_{RM} value ($\Delta_m \leq U_\Delta$) and that the difference between the values determined in the laboratory and the RM value was not greater than $\pm 10\%$, the AA extraction can be applied under the two variants.

Considering that in V7 the number of additional steps is reduced (water heating and temperature measurement) compared to V3, for AA extraction the water extraction variant at room temperature (V7) will be selected.

Similar results were obtained by Saraji and Javadian (2019) who studied different temperatures for AA extraction ranging from 15 to 50°C and showed that the optimum extraction efficiency was obtained when the extraction took place at room temperature (25°C).

Table 3: Effect of different extraction factors on AA content

Variable factors	C_V (mean $\pm$ SD), $\mu\text{g/kg}$	C_{RM} , $\mu\text{g/kg}$	Required conditions	
			Dif (%) $\leq \pm 10\%$	$\Delta_m \leq U_\Delta$
Water temperature 60°C (V3)	672.48 $\pm$ 20.21	625	+7.58 $\leq \pm 10\%$	47.37 $\leq$ 53.31
Room temperature (V7)	658.57 $\pm$ 23.13		+5.37 $\leq \pm 10\%$	33.57 $\leq$ 52.63
Hexane volume 10 mL (V7)	615.99 $\pm$ 35.83		−1.44 $\leq \pm 10\%$	9.01 $\leq$ 51.64
5 mL (V8)	647.17 $\pm$ 29.25		+3.55 $\leq \pm 10\%$	22.17 $\leq$ 50.14
Purification with Carrez (V7)	647.49 $\pm$ 29.24		3.60 $\leq \pm 10\%$	22.49 $\leq$ 52.05
Without Carrez (V9)	642.68 $\pm$ 46.02		+2.83 $\leq \pm 10\%$	17.68 $\leq$ 55.53

Some authors reported that AA extraction from cereal-based products was realized with water at room temperature (Pittet et al. 2004), while others (Dunovská et al. 2004) recommended hot water (60–80°C) for AA extraction from potato chips, as AA is stable at this temperature and the extraction efficiency can increase.

3.1.3 The volume of hexane used for defatting the sample

AA is practically insoluble in non-polar organic solvents such as hexane, heptane, and carbon tetrachloride. Because potato-based products uptake oil when fried, the obtained emulsion can influence the purification procedure. As a consequence, it can result in an overlapped peak with the analyte of interest, so defatting the matrices in parallel with the extraction step was performed. These experiments were realized with different volumes of hexane: 10 mL (V7) and 5 mL (V8).

The results obtained after comparing the mean concentration values determined under the conditions of variants V7 and V8 with the RM concentration value showed that the required conditions are accomplished, with no significant differences between the values (Linsinger 2010). It was observed that when using a volume of 10 mL hexane, the AA extracted in water was less colored, the cleaned-up extract on SPE cartridges was suitable compared to V8, vacuum not being necessary in the extraction procedure. The fat was better removed when a higher amount of hexane was used (V7), thus reducing the purification time of the extract by the SPE procedure. For these reasons, the conditions of the V7 variant will be considered optimal for AA extraction. Cheng et al. (2019) studied the fat extraction efficiency of hexane, cyclohexane, and toluene from fried samples and from all these solvents, hexane was chosen as the best defatting solvent. Also, Bermudo et al. (2006) as well as Fernandes and Soares (2007) stated

that the fat removal with *n*-hexane from different food products, such as cereal-based products, French fries, and potato chips, ensures a more adequate contact with the water incorporated into the food matrix, finally improving the extraction efficiency of AA.

3.1.4 Influence of chemical purification of extracts with Carrez solutions

In order to achieve a clear extract, the chemical purification of aqueous phase of AA collected after extraction, homogenization, and centrifugation was treated with Carrez solutions (V7). The V9 without Carrez was also realized, as shown in Table 1. By adding potassium hexacyanoferrate trihydrate solution, $K_4[Fe(CN)_6] \cdot 3H_2O$ (Carrez I), followed by a heptahydrate zinc sulfate solution, $ZnSO_4 \cdot 7H_2O$ (Carrez II), the proteins can be removed to the extent of 85% (Sun et al. 2003; Soares 2015). For both variants performed under the working conditions mentioned, the required conditions were accomplished, concluding that between the C_V determined and the C_{RM} there are no significant differences. The difference between the two variants is the fact that the experiment without Carrez solutions (V9) is less expensive, requiring a shorter preparation time, the need for less reagents, and therefore further experiments will be performed with the V9 conditions. Addition of Carrez solutions was also reported by Ciesarová et al. (2004), Gökmen et al. (2005), and Şenyuva and Gökmen (2005) for AA determination in potato chips and crisps and other cereal products.

A maximum efficiency of AA extraction from the matrix and a sufficiently cleaned-up extract were obtained when the extraction and purification of the AA extracts from the potato products were performed with water at room temperature, simultaneously with 10 mL hexane, without Carrez solutions, and the clean-up was realized on two SPE cartridges, using an optimum elution solvent volume of 5 mL (V9).

Figure 2: Chromatogram of chips (a) and French fries (b) samples obtained with the FC procedure. AA – acrylamide peak, IS – labeled AA peak.

3.1.5 Evaluation of some validation parameters of the SPE procedure

Under the optimized extraction condition of SPE procedure, some validation parameters were evaluated. In terms of linearity, the calibration curves were linear over the two concentration ranges of 50–500 µg/L and 400–5,000 µg/L, respectively, with the correlation coefficient $R^2 > 0.9992$. The RSD for repeatability ranged between 2.05 and 4.21%. The interlaboratory reproducibility was evaluated by the z-score

of –0.8, obtained by participation in the PT 3095 on French fries test material launched by the FAPAS program. The sensitivity of the method was evaluated by LOD and LOQ. A LOD of 6.94 µg/kg and a LOQ of 20.83 µg/kg, respectively, were obtained for the SPE procedure, fulfilling the required conditions set by the European Commission (2017). The recovery for the spiked samples ranged between 85.64 and 104.51%. The analysis of results showed that the newly developed method is characterized by both high precision and accuracy.

Figure 3: Chromatogram of chips (a) and French fries (b) samples obtained with the SPE procedure developed. AA – acrylamide peak, IS – labeled AA peak.

3.2 Functionality of the developed method

3.2.1 Comparative study of the SPE and the FC procedures

AA concentration of potato-based samples (chips and French fries) was determined, based on both procedures described, FC procedure (Figure 2) and by applying the optimum working conditions of the SPE clean-up procedure (Figure 3). The obtained results are presented in Table 4.

As it can be seen in Table 4, for all analyzed samples, the extended uncertainty (U_{Δ}) is higher than the absolute difference (Δ_m), which means that there is no significant difference between the AA content obtained by using the FC and SPE procedures.

The procedures were compared by using a regression line and the results are shown in the graph from Figure 4, where on x axis are presented the results obtained by using the FC procedure, while on y axis are presented the results obtained by using the SPE procedure. By analyzing

Table 4: Levels of AA determined in the samples analyzed using both procedures (FC, SPE)

Samples	Acrylamide (mean $\pm$ SD), $\mu\text{g/kg}$				Required conditions		
	FC	n	SPE	n	Δ_m	U_{Δ}	$\Delta_m \leq U_{\Delta}$
Chips brand 1	163.63 $\pm$ 2.83 (Figure 2)	3	155.84 $\pm$ 4.10 (Figure 3)	8	11.13	19.19	T
Chips brand 2	179.71 $\pm$ 4.04	3	165.27 $\pm$ 6.54	14	14.44	15.82	T
Chips brand 3	326.73 $\pm$ 12.01	3	308.57 $\pm$ 10.30	7	18.15	41.04	T
Chips brand 4	902.87 $\pm$ 31.52	3	867.09 $\pm$ 43.98	9	35.78	241.18	T
French Fries (precooked)	65.97 $\pm$ 1.58	6	62.40 $\pm$ 1.43	3	3.57	13.00	T
French fries – fast food 1	212.19 $\pm$ 5.57	3	198.00 $\pm$ 2.09	3	14.19	40.23	T
French fries – fast food 2	222.51 $\pm$ 2.94	3	203.68 $\pm$ 1.35	3	18.83	41.38	T
French fries – fast food 3	394.08 $\pm$ 6.55 (Figure 2)	3	371.30 $\pm$ 2.06 (Figure 3)	3	22.78	75.19	T

n – number of replicates; T – true.

Figure 4: Graphical comparison of the two procedures used to extract AA from chips and French fries samples

Table 5: PTs results

PTs	Matrix	Assigned value ($\mu\text{g/kg}$)	Results ($\mu\text{g/kg}$)		z-Score	
			FC	SPE	FC	SPE
FAPAS 3094	Biscuit (cookie)	293	286.79*	273.66**	−0.1	−0.3**
FAPAS 3095	French Fries (precooked)	75.4	65.97**	62.40*	−0.3**	−0.8

*declared value to FAPAS realized with the procedure indicated. **Values obtained on the same matrix, processed in parallel with the indicated procedure; z values are calculated based on the formula $z = \frac{(x - x_a)}{\sigma_p}$, where x is the result obtained, x_a is the assigned value, and σ_p is the standard deviation for proficiency.

the AA content determined by using these procedures, it can be concluded that there is a close similarity between results ($R^2 = 0.9999$).

Considering that there are no significant differences ($\Delta_m \leq U_\Delta$) between results obtained by using the SPE and FC procedures, the new procedure developed can be used with confidence for AA determination from potato-based products.

Application of SPE cartridges in sample preparation allows the simultaneous extraction and clean-up of analyte, reduction of sorbent quantities (31%), solvent volumes (21%), amount of reagents (13% KBr; 10% bromine water), and the preparation time (50%), compared to the previous method of extraction and purification (FC), which is time consuming, and laborious involving several stages of manual handling (calcination, florisil weighing and activation, preparation and washing of chromatographic columns for purification, etc.), thus resulting in a cost reduction with ~30%.

3.2.2 PTs

The functionality and accuracy of the results of the new SPE procedure were demonstrated by participation in 2019 in two PTs launched by the FAPAS program: FAPAS 3094 (AA in biscuit-cookie) with the FC procedure and FAPAS 3095 (AA in French fries–precooked) with the SPE procedure. The RMs received were processed by both procedures, yielding z-scores less than ± 2 (Table 5).

The obtained results demonstrate the accuracy of the SPE procedure developed.

4 Conclusions

The present study reports a new approach in the sample preparation to determine the AA content by GC-MS/MS from potato-based products using the SPE procedure. A maximum efficiency of analyte extraction from chips and French fries samples and a sufficiently cleaned-up extract were obtained when the extraction and clean-up of the AA extracts from these matrices were performed with water at room temperature, simultaneously with 10 mL hexane, without Carrez solutions, and the clean-up was realized on two SPE cartridges, using an optimum elution solvent volume of 5 mL.

The proficiency test results (z-score: -0.8) of the FAPAS program suggested that the present SPE developed method could be applied for routine analysis in

order to guarantee the quality control of AA in cereal and potato products.

Abbreviations

AA	acrylamide
C_{RM}	certified concentration value of the reference material
C_V	concentration value of the reference material determined in the experimental variant
FC	florisil column
LOD	limit of detection
LOQ	limit of quantification
$RSD_{(R)}$	reproducibility relative standard deviation
SPE	solide-phase extraction

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References

- [1] Bermudo E, Núñez O, Puignou L, Galceran MT. Analysis of acrylamide in food samples by capillary zone electrophoresis. *J Chromatogr A*. 2006;1120:199–204. doi: 10.1016/j.chroma.2005.10.074.
- [2] Cheng WC, Hsiao SW, Chou SS, Sun-Hwang L, Lu TJ, Yeh AI. Determination of acrylamide in Chinese foods by GC-ion trap MS using 2-bromopropenamide and 2-bromopropenamide- $^{13}C_3$. *J Food Drug Anal*. 2006;14(2):207–14.
- [3] Cheng J, Zhang S, Wang S, Wang P, Su X-O, Xie J. Rapid and sensitive detection of acrylamide in fried food using dispersive solid-phase extraction combined with surface-enhanced Raman spectroscopy. *Food Chem*. 2019;276:157–63. doi: 10.1016/j.foodchem.2018.10.004.
- [4] Ciesarová Z, Balasová V, Kiss E, Kolek E, Šimko P, Kováč M. Comparison of two methods for acrylamide determination and dietary intake of acrylamide from potato crisps in Slovakia. *Czech J Food Sci*. 2004;22:251–4.
- [5] Dias FFG, Bogusz Junior S, Hantao LW, Augusto F, Sato HH. Acrylamide mitigation in French fries using native L-asparaginase from *Aspergillus oryzae* CCT 3940. *LWT-Food Sci Technol*. 2017;76:222–9. doi: 10.1016/j.lwt.2016.04.017.
- [6] Dunovská L, Hajšlová J, Čajka T, Holadová K, Hájková K. Changes of acrylamide levels in food products during technological processing. *Czech J Food Sci*. 2004;22:283–6.

- [7] European Commission (EU). Commission Regulation 2017/2158 of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. Official J Eur Union. 2017;L304:24–44. Available from: <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R2158&from=EN>.
- [8] European Food Safety Authority (EFSA). Scientific Opinion on acrylamide in food. EFSA Panel on Contaminants in the Food Chain. EFSA J. 2015;13(6):4104, Parma, Italy. Available from: <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2015.4104>.
- [9] Fernandes JO, Soares C. Application of matrix solid-phase dispersion in the determination of acrylamide in potato chips. J Chromatogr A. 2007;1175:1–6. doi: 10.1016/j.chroma.2007.10.030.
- [10] Food Drink Europe. Acrylamide toolbox; 2019. Available from: https://www.fooddrinkEurope.eu/uploads/publications_documents/FoodDrinkEurope_Acrylamide_Toolbox_2019.pdf.
- [11] FRD Center – Food market in Romania; 2016. Available from: <https://www.dutchromaniannetwork.nl/wp-content/uploads/2017/01/Food-Report-Romania-2016.pdf>.
- [12] Galuch MB, Magon TFS, Silveira R, Nicácio AE, Pizzo JS, Bonafe EG, et al. Determination of acrylamide in brewed coffee by dispersive liquid-liquid microextraction (DLLME) and ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). Food Chem. 2019;282:120–6. doi: 10.1016/j.foodchem.2018.12.114.
- [13] Genovese J, Tappi S, Luo W, Tylewicz U, Marzocchi S, Marziali S, et al. Important factors to consider for acrylamide mitigation in potato crisps using pulsed electric fields. Innov Food Sci Emerg Technol. 2019;55:18–26. doi: 10.1016/j.ifset.2019.05.008.
- [14] Ghiasvand AR, Hajipour S. Direct determination of acrylamide in potato chips by using headspace solid-phase extraction microextraction coupled with gas chromatography-flame ionization detection. Talanta. 2016;146:417–22. doi: 10.1016/j.talanta.2015.09.004.
- [15] Gökmen V, Şenyuva HZ, Acar J, Sarioğlu K. Determination of acrylamide in potato chips and crisps by high-performance liquid chromatography. J Chromatogr A. 2005;1088(1–2):193–9. doi: 10.1016/j.chroma.2004.10.094.
- [16] Hoenicke K, Gattermann R, Harder W, Hartig L. Analysis of acrylamide in different foodstuffs using liquid chromatography-tandem mass spectrometry and gas chromatography-tandem mass spectrometry. Anal Chim Acta. 2004;520(1–2):207–15. doi: 10.1016/j.aca.2004.03.086.
- [17] International Agency for Research on Cancer (IARC). Monographs on the evaluation of carcinogenic risks of chemicals to humans. Lyon, France; 1994. vol. 60, p. 389.
- [18] Lambert M, Inthavong C, Hommet F, Leblanc J-C, Hulin M, Guérin T. Levels of acrylamide in foods included in ‘the first French total diet study on infants and toddlers’. Food Chem. 2018;240:997–1004. doi: 10.1016/j.foodchem.2017.08.035.
- [19] Linsinger T. European Reference Materials (ERM). Application note 1. Comparison of a measurement result with the certified value. 2010. p. 1–2.
- [20] Liu J, Zhao G, Yuan Y, Chen F, Hu X. Quantitative analysis of acrylamide in tea by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. Food Chem. 2008;108:760–7. doi: 10.1016/j.foodchem.2007.11.015.
- [21] Negoită M, Culeţu A. Application of an accurate and validated method for identification and quantification of acrylamide in bread, biscuits and other bakery products using GC-MS/MS system. J Braz Chem Soc. 2016;27(10):1782–91. doi: 10.5935/0103-5053.20160059.
- [22] Nematollahi A, Kamankesh M, Hosseini H, Ghasemi J, Hosseini-Esfahani F, Mohammadi A. Investigation and determination of acrylamide in the main group of cereal products using advanced microextraction method coupled with gas chromatography-mass spectrometry. J Cereal Sci. 2019;87:157–64. doi: 10.1016/j.jcs.2019.03.019.
- [23] Pittet A, Périsset A, Oberson J-M. Trace level determination of acrylamide in cereal-based foods by gas chromatography-mass spectrometry. J Chromatogr A. 2004;1035:123–30. doi: 10.1016/j.chroma.2004.02.037.
- [24] Sanny M, Jinap S, Bakker EJ, van Boekel MAJS, Luning PA. Is lowering reducing sugars concentration in French fries an effective measure to reduce acrylamide concentration in food service establishments? Food Chem. 2012;135:2012–20. doi: 10.1016/j.foodchem.2012.06.052.
- [25] Saraji M, Javadian S. Single-drop microextraction combined with gas chromatography-electron capture detection for the determination of acrylamide in food samples. Food Chem. 2019;274:55–60. doi: 10.1016/j.foodchem.2018.08.108.
- [26] Şenyuva HZ, Gökmen V. Survey of acrylamide in Turkish foods by an in-house validated LC-MS method. Food Addit Contam. 2005;22:204–9. doi: 10.1080/02652030512331344178.
- [27] Soares CMD. Assessment of the dietary intake of acrylamide in Portugal. Development and evaluation of strategies for reduction of acrylamide formation in thermally processed foods. Porto: Universidade do Porto; 2015. Available from: <https://pdfs.semanticscholar.org/bc8f/f5e31edfd5647523b6922a035450db3193f8.pdf>.
- [28] SR EN 16618:2015 Food analysis. Determination of acrylamide in food by liquid chromatography tandem mass spectrometry (LC-ESI-MS/MS).
- [29] Sun J, Zhang X, Broderick M, Fein H. Measurement of nitric oxide production in biological systems by using Griess reaction assay. Sensors. 2003;3:276–84.
- [30] U.S. Environmental Protection Agency (EPA). Toxicological review of acrylamide. In support of summary information on the integrated risk information system (IRIS). EPA/635/R-07/009F; 2010. Available from: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0286tr.pdf.
- [31] U.S. National Toxicology Program (NTP). Department of health and human service. Center for the evaluation of risks to human reproduction. NTP-CERHR monograph on the potential human reproductive and developmental effects of acrylamide; 2005. NIH publication no. 05-4473. Available from: https://ntp.niehs.nih.gov/ntp/ohat/acrylamide/acrylamide_monograph.pdf.
- [32] Yang Y, Achaerandio I, Pujolà M. Influence of the frying process and potato cultivar on acrylamide formation in French fries. Food Control. 2016;62:216–23. doi: 10.1016/j.foodcont.2015.10.028.
- [33] Zhang Y, Zhang G, Zhang Y. Occurrence and analytical methods of acrylamide in heat-treated foods. J Chromatogr A. 2005;1075:1–21. doi: 10.1016/j.chroma.2005.03.123.