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Straightforward and rapid determination of acrylamide in coffee beans by means of HS-SPME/GC-MS

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ABSTRACT

A straightforward and rapid preparation procedure for the extraction of the acrylamide from coffee beans, by means of the volatile silylated derivatives of acrylamide (AA) and headspace solid phase microextraction (HS-SPME) is described. Commercially available SPME fibre coated with polydimethylsiloxane (PDMS) was used. The silylation reactions of the AA were executed with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). The deuterium labelled d_3-acrylamide was used as an internal standard. The acrylamide level was quantified using gas chromatography coupled with the mass spectrometry (GC-MS) in the selected ion monitoring (SIM) mode. The limit of quantification (LOQ) for measuring acrylamide was 3 µg/kg. The described method demonstrates satisfactory repeatability (RSD = 2.6%), with an intermediate precision (RSD = 9.4%) and recovery (99–105%).

1. Introduction

The acrylamide is formed during the thermal processing (i.e. frying and baking) of carbohydrate-rich foods as one of the products of the Maillard reaction, in the reaction between asparagine and sugars (glucose and fructose) (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002). It occurs mainly in foods such as potato products (fries and chips), cereal products (bread, cereals, biscuits) as well as coffee and coffee substitutes (Alves, Soares, Casal, Fernandes, & Oliveira, 2010). Due to reports of neurotoxic, genotoxic and carcinogenic activity of acrylamide, the presence of this compound in food may pose a risk to human health (Bergmark, 1997; Bull et al., 1984; Chapin et al., 1995; Friedman, Dulak, & Stedman, 1995; Johnson et al., 1986; Lehning, Persaud, Dyer, Jortner, & LoPachin, 1998; LoPachin, 2004; Tyl, Marr, Myers, Ross, & Friedman, 2000). The International Agency for Research on Cancer in 1994 classified acrylamide as "probably carcinogenic to humans" (group 2A) (International Agency for Research on Cancer, 1994). The European Food Safety Authority (EFSA) confirmed in 2015, that acrylamide is a carcinogen (EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain) (2015), 2015). Due to the toxic effect of acrylamide on human health, European Commission has issued a recommendation to monitor, in all Member States, the acrylamide content in selected groups of the food products. The latest European Commission regulation on the monitoring of acrylamide in food has been published on November 20, 2017 and a reference levels of acrylamide was set at 400 µg/kg for roasted coffee whilst for instant coffee this was 850 µg/kg (Commission Regulation (EU) 2017/2158, 2017). According to the above, many analytical methods have been developed to determine of acrylamide in food among others in coffee, which is one of the most popular beverages in the world.

The commonly used methods are based on the gas chromatography (GC) or high performance liquid chromatography (HPLC) coupled with mass spectrometric detection (GC-MS and LC-MS) (Andrzejewski, Roach, Gay, & Musser, 2004; Castle & Eriksson, 2005; Mastovska & Lehotay, 2006; Soares, Cunha, & Fernandes, 2006; Soares, Alves, Casal, Oliveira, & Fernandes, 2010; Wenzl, Szilagyi, Rosén, & Karasek, 2009; Zhang, Zhang, & Zhang, 2005). The LC-MS method requires a sample purification step, most often by using solid phase extraction (SPE) while GC-MS requires obtaining an acrylamide derivative, usually the bromide derivative. For example the SPE purification method has been used by Bortolomeazzi for the determination of acrylamide in roasted coffee by liquid chromatography tandem mass spectrometry (Bortolomeazzi, Munari, Anese, & Verardo, 2012).

In recent years, the solid phase microextraction (SPME) method has become very popular in food analysis. Cagliero et al. used direct immersion solid-phase microextraction (DI-SPME) coupled with GC-MS to determine acrylamide in brewed coffee and coffee powder (Cagliero, Ho, Zhang, Bicchi, & Anderson, 2016). HS-SPME/GC-MS method for analysis of acrylamide in food was used also by Lagalante & Felter (2004). Moreover, the authors claimed, that silylation of acrylamide offers a preferable method over bromination. The need to monitor the levels of acrylamide in coffee makes that the methods used should be

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both accurate and relatively inexpensive. In this paper, we propose a modified procedure enabling determination of acrylamide in coffee beans, less expensive than previously described. The procedure described in this article is based on the HS-SPME/GC-MS method developed by Lagalante with some modification e.g. deuterium labelled d_3-acrylamide was used as internal standard.

2. Materials and methods

2.1. Chemical and materials

Acrylamide (Sigma-Aldrich, USA, 99% purity), d_3-acrylamide-2,3,3 (d_3-AA) standard solution 500 mg/L in acetonitrile (Sigma-Aldrich, USA, 98% purity) and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Supelco, USA) were stored at 4 °C. The solutions were prepared with anhydrous acetonitrile (Sigma-Aldrich, USA 99.8% purity). The calibration solutions were refrigerated at 4 °C for a maximum of 4 weeks. Grains of unroasted coffee Arabica and Robusta as well as roasted coffee samples were obtained from a local supermarket. All samples were hermetically packed and keep at 4 °C.

2.2. Silylation of acrylamide

The silylation reactions were prepared using volumetric pipets dispensed directly to the 1.5 mL screw-top vials with PTFE/silicone septa (Lab Logistic Group GmbH, Germany). However, BSTFA was added through a septum using 100 µL glass syringe (Hamilton, USA). Due to the selected capacity of the vials and the headspace analysis, it was decided that an optimal amount of solution would be 250 µL. Based on the literature data, the range of the calibration curve was determined up to 5000 µg/kg in relation to the internal standard (d_3-acrylamide-2,3,3) with a concentration of 1000 µg/kg for all calibration levels. The mixing and heating process of the vial was carried out using a TriPLUS RSH autosampler.

2.3. HS-SPME/GC-MS analysis

The samples were analysed on a Trace 1310 GC equipped with a TriPLUS RSH autosampler (Thermo Fisher Scientific, USA) with mounted SPME holder (Thermo Fisher Scientific, USA) equipped with 100 µm polydimethylsiloxane (PDMS) fibre coupled to a ISQ QD mass spectrometer. Before carrying out the measurements and after each analysis, the fibre was conditioned in accordance with the manufacturer's recommendations. The GC-MS were controlled by Excalibur software (Thermo Fisher Scientific, USA). Due to the applied SPME technique, splitless injection mode and liner 0.8 mm i.d. were used. Separation was achieved with a column of 007-5MS, 30 m length × 0.25 mm ID, 0.25 µm film thickness (Quadrex, USA). Helium was used as a carrier gas with a column flow rate of 1.0 mL min^{-1}. Temperature program: 50 °C, ramp 6 °C min^{-1} to 110 °C, ramp 25 °C min^{-1} to 270 °C, hold for 6 min was applied. The mass selective detector was operated at 70 eV in the EI mode over the *m/z* range 5–400, scanned at 0.2 s intervals and temperature of transfer line 250 °C. Due to the high concentration of unreacted BSTFA in the analysed sample, the signal was collected after 7 min. The signal from the determined compound and the internal standard was recorded at 9.47 min of analysis.

2.4. Analytical method validation

The validation of the method was based on the following parameters: linearity, selectivity, precision and accuracy, and the HORRAT coefficient value, which is increasingly common in standards. The method's linearity was estimated from values determined for the calibration levels in a calibration curve. The selectivity of the method was determined based on the comparison of the results obtained in a model solution prepared in acetonitrile, with the results of real samples. The precision of the method is described by means of repeatability – for measurements performed during one day and intermediate precision – for measurements conducted over the course of several days. Both parameters were evaluated by analyzing the green unroasted coffee spiked at 3 µg/kg acrylamide. Calculations of the above mentioned parameters involved 3 independent measurements in the same day for repeatability and 6 independent measurements over two consecutive days for intermediate precision. The parameters were evaluated using the same method, reagent and equipment, which was operated by the same analyst. Repeatability as well as intermediate precision were expressed in percent, as a relative standard deviation (RSD).

The HORRAT coefficient value was used to characterize the precision of the method, which is the RSD ratio calculated from the value of the test sample parameter to the RSD calculated from the Horwitz formula:

$$RSD = 2^{(1-0.5\log C)} \cdot 0.67$$

where: RSD – relative standard deviation, C – analyte concentration.

The method accuracy assessments were made by determining recovery. The tests were carried out using three levels (100, 500 and 2500 µg/kg) of spiked acrylamide.

The acrylamide was quantified by the method of internal standards using d_3-acrylamide-2,3,3. Due to the fact, that the compound to be determined does not show the required volatility, the procedure of derivatization was used. The derivatization of acrylamide was carried out using BSTFA, obtaining *N,O*-bis(trimethylsilyl)acrylamide (BTMSAA) and d_3-*N,O*-bis(trimethylsilyl)acrylamide (d_3-BTMSAA), respectively. The identification of compounds was carried out on the basis of the mass spectra recorded in the total ion current (TIC) mode. The quantitative analysis was performed on the basis of area peak a characteristic ion for BTMSAA and d_3-BTMSAA recorded in the selected ion monitoring (SIM) mode.

The calibration curves were made in the range from 3 to 5000 µg/kg terms of 1000 µg/kg of an internal standard. Due to the lack of a certified reference material, the quantitative results were verified on the basis of coffee recovery experiments.

A percentage recovery experiment was undertaken using the commercial coffee beans. For this purpose, the level of the AA in coffee beans matrix was determined before and after its spike in the amount of 100, 500 and 2500 µg/kg. The portions of 3 g of coffee beans were ground to powder in a dedicated mill to coffee beans. The 1 g of coffee powder sample was spike of AA. Next, 10 mL of acetonitrile and 1000 µg/kg of d_3-AA as internal standard were added and stirred in 20 mL vial for 1 h in an ambient temperature. An aliquot of the supernatant acetonitrile phase was transferred to a 1.5 mL Eppendorf tube and centrifuged in MPW-52 (MPW Med. Instruments, Poland) at 5400 g for 10 min. A 250 µL of centrifuged sample was transferred by a pipette to 1.5 mL vial and derivatization by using optimized silylation procedure has been applied. In the case of a spiked sample, to 1 g of a sample 100, 500 or 2500 µg/kg AA was added. The acrylamide levels in the natural and spiked samples were analysed in triplicate.

3. Results

3.1. Optimization of qualitative and quantification analysis on a mass spectrometer

The observed mass spectral fragmentation pattern for derivatization product of AA and d_3-AA is shown in Fig. 1A and B. The intensity of the molecular ion (M^+) for silylated AA at *m/z* 200 and d_3-AA at *m/z* 203 obtained by 70 eV ionization energies is very small and cannot be used for quantitative analysis. For this reason, the most intense ion *m/z* 128 for BTMSAA and *m/z* 131 for d_3-BTMSAA was selected for quantifying acrylamide levels. The confirmatory identification was shown on the basis of ion *m/z* 200 for BTMSAA and *m/z* 203 for d_3-BTMSAA,

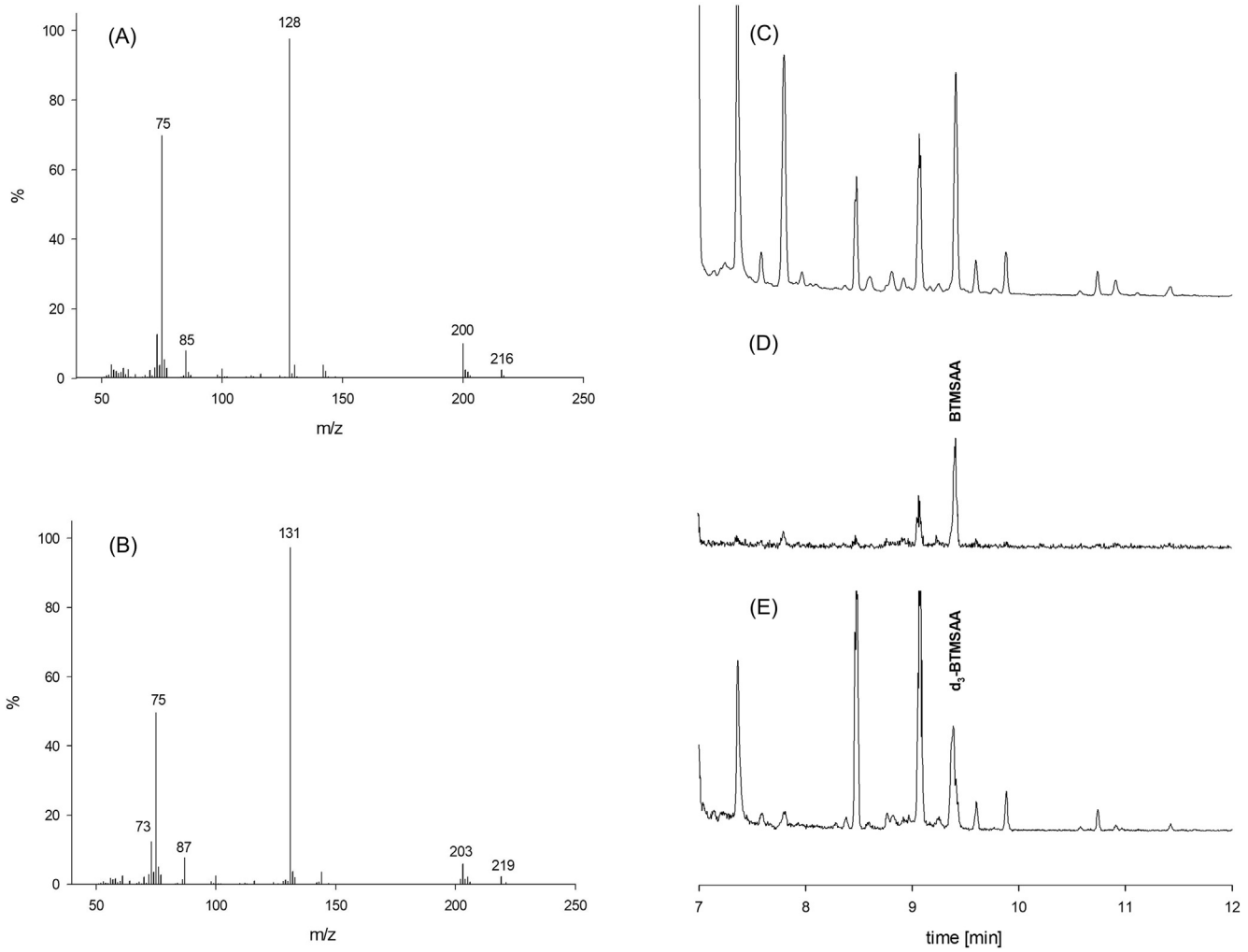

Fig. 1. The observed mass spectra for (A) BTMSAA, (B) d_3-BTMSAA and (C) the total ion current chromatogram, (D) selected ion monitoring chromatogram of *m/z* 128 for BTMSAA, (E) selected ion monitoring chromatogram of *m/z* 131 for d_3-BTMSAA.

respectively. The selected ion fragment was recorded in the SIM mode to maximize analytical sensitivity. The resultant TIC and SIM chromatograms obtained for green unroasted coffee spiked 10 µg/kg AA and 10 µg/kg d_3-AA are shown in Fig. 1C–E. The chromatograms confirm the absence of ions coming from co-eluting compounds, which allows for quantitative analysis.

3.2. Optimization of the acrylamide silylation conditions

The acrylamide is low volatility and polarity compound of high water solubility. Therefore, in order to perform HS-SPME analysis it is necessary to carry out derivatization. This process pursued to decrease the polarity of acrylamide and increase the fibre-headspace and headspace-solution partition during SPME sampling. Due to the nature of the matrix, which is coffee, the study was decided to run on a model solution that contained 1000 µg/kg AA and 1000 µg/kg d_3-AA in anhydrous acetonitrile. The effect of the amount of BSTFA added, the temperature of the process and the time of reaction will have on the silylation process has been checked. The tests were carried out in closed PTFE membranes/silicone vials with a capacity of 1.5 mL. Due to the selected capacity of the vials and the headspace analysis, it was decided that an optimal amount of the model solution for testing would be 250 µL. To determine the optimal amount of BSTFA 5, 10, 20 and 30%, BSTFA to the acrylamide solution was added. To determine adequate the temperature of the silylation process, the process was carried out at 20, 50, 70 and 80 °C; using a 20% V/V BSTFA and a 60 min reaction time. In order to determine the influence of the process time on the amount of silyl derivative obtained, this process was carried out on the basis of 10, 20, 40 and 60 min of reaction time; using a 20% V/V BSTFA and a 70 °C temperature of the process. Each measurement was repeated three times. The results of the study are presented in Fig. 2A–C. It shows that in order to obtain the best performance of the reaction, the process should be carried out for 1 h at 70 °C at 20% volume of BSTFA in relation to the volume of the acrylamide solution.

3.3. Optimization of sorption/desorption conditions

The conditions of the sorption/desorption process on the SPME fibre were also optimized. It was analysed how time and temperature affect the process. Before study, samples were prepared according to the optimal conditions of the silylation process described above. In order to determine the optimal sorption temperature on the SPME fibre, the sorption process was carried out for 30 min at 30, 40, 50, 60, 70 °C. The correct sorption process time is based on the sorption process at 70 °C and was carried out in 2.5, 5, 10, 15 and 20 min. The optimal desorption temperature on the SPME fibre was determined by process carried out for 2 min at 210, 230 and 250 °C. The optimal time of the desorption process was determined based on the measurements made at 250 °C for: 1, 2, 5 and 10 min. The collected results are presented in Fig. 3A and B. It shows that the best results for sorption process on

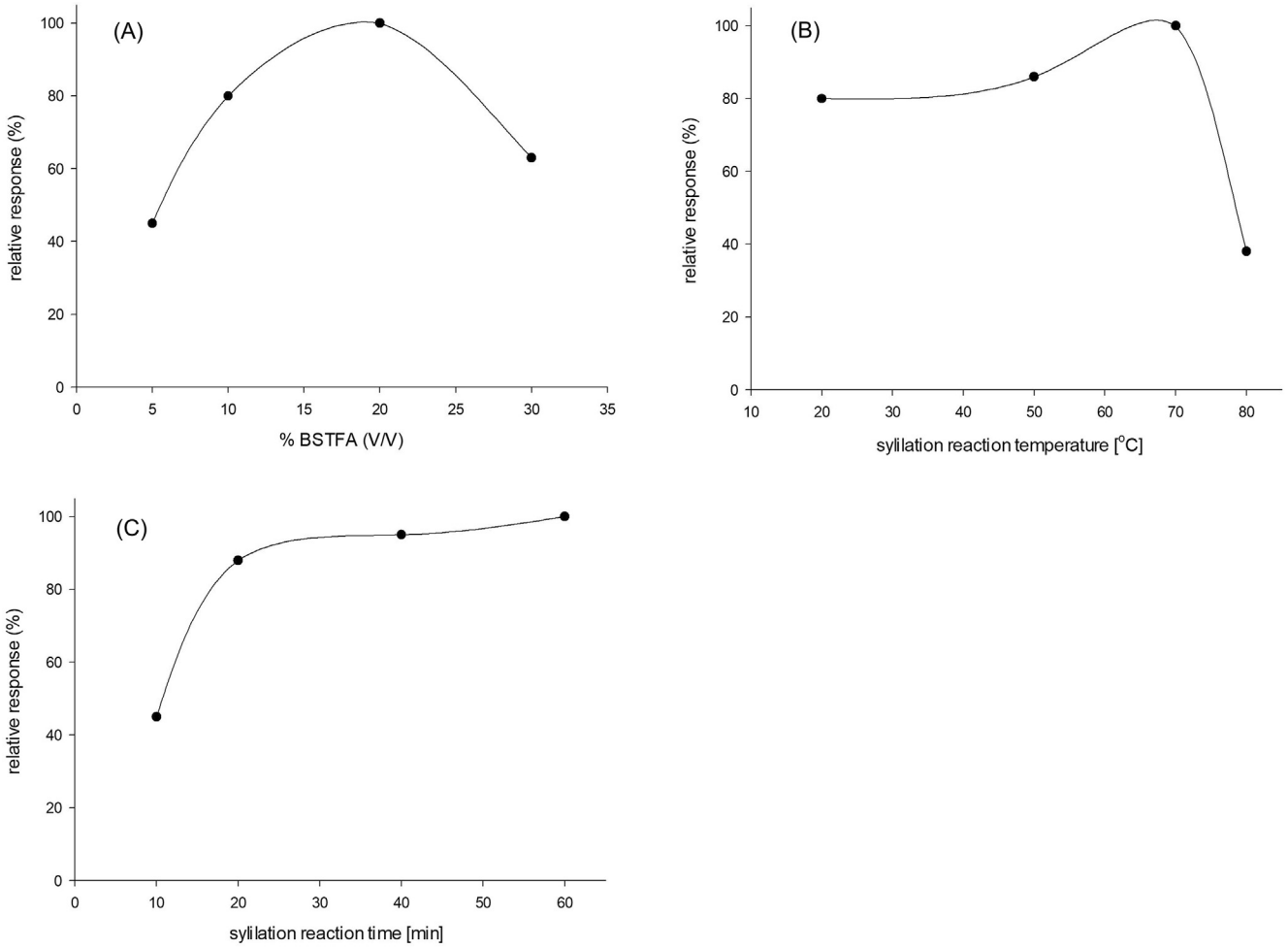

Fig. 2. Optimization of the acrylamide silylation conditions: (A) optimization V/V BSTFA – silylation conditions: 70 °C, 60 min; (B) optimization temperature reaction – silylation conditions: 20% V/V BSTFA, 60 min; (C) optimization reaction time – silylation conditions: 20% V/V BSTFA, 70 °C.

SPME fibre are obtained for 10 min at 60 °C. However, due to the fact that the silylation reaction and the sorption process were carried out in the TriPlus RSH autosampler system in both cases, the same temperature was used: 70 °C. The obtained data show that to maximize the sensitivity of the developed method, the desorption process should be performed at 250 °C (Fig. 3C) – the maximum temperature recommended by the fibre manufacturer and with a long desorption time of 10 min (Fig. 3D). However, due to the observed jump of the chromatographic signal associated with removing the fibre from the injector, it was necessary to shorten the desorption time. Then the optimal value of the time appeared to be 2 min.

3.4. Analytical method validation. Preparation of the calibration curve, determination of the detection limit and recovery

The quantitative analysis was carried out using the internal standard method, using the deuterium derivative of acrylamide. The calibration curves (Fig. 4) were prepared on the basis of the ratio of the peak area recorded in the SIM mode for the silylated acrylamide (*m/z* 128) to the silylated peak area of the internal standard (*m/z* 131).

The calibration curve has been determined for two ranges: low concentrations (3–150 µg/kg) for 6 points and high concentrations (150–5000 µg/kg) for 7 points. The mean intensity values for points on the calibration curve were calculated on the basis of replicates of 3 measurements. To assess the quality of the obtained calibration curves r values were calculated and were equal to 0.99971 and 0.99980 for low and high concentrations, respectively. Other parameters such as a slope of the line and y-intercept are in Table 2. For mathematical verification of linearity Mandel's fitting test was used. The residual is defined as the difference between the measured value of the analytical signal and the value calculated, based on the found equation of calibration curve. Mandel's fitting test is based on a comparison the standard error of a straight-line regression model and the standard error of a second-order polynomial regression model. The found values of F are lower than the critical value, therefore the calibration curves can be considered as linear in the examined concentration ranges, at the 95% confidence level (Brüggemann, Quapp, & Wennrich, 2006). Determination coefficients, analysis of residuals and significance test showed that calibration curves were linear in selected concentration ranges. These values indicate that the obtained curve can be successfully used for quantitative analysis. Based on the ratio of the analytical signal generated by a spiked of acrylamide in acetonitrile to the noise (S/N) as the limit of detection (LOD) (S/N = 3) 1 µg/kg was assumed and the limit of quantification (LOQ) (S/N = 10) 3 µg/kg was taken into account.

Repeatability and intermediate precision were checked for green unroasted coffee spiked acrylamide with level of corresponding to LOQ (for repeatability the RSD = 2.6% and for intermediate precision the RSD = 9.4%). Parameters of HS-SPME/GC-MS method for the acrylamide determination in the green unroasted coffee spiked acrylamide are presented in Table 1.

Furthermore, the repeatability was determined by analysing samples of roasted coffee, 3 replicates in parallel (Table 2). The procedure was based on determining the recovery and was carried out on four commercial coffee samples. Coffee samples were different in the grain

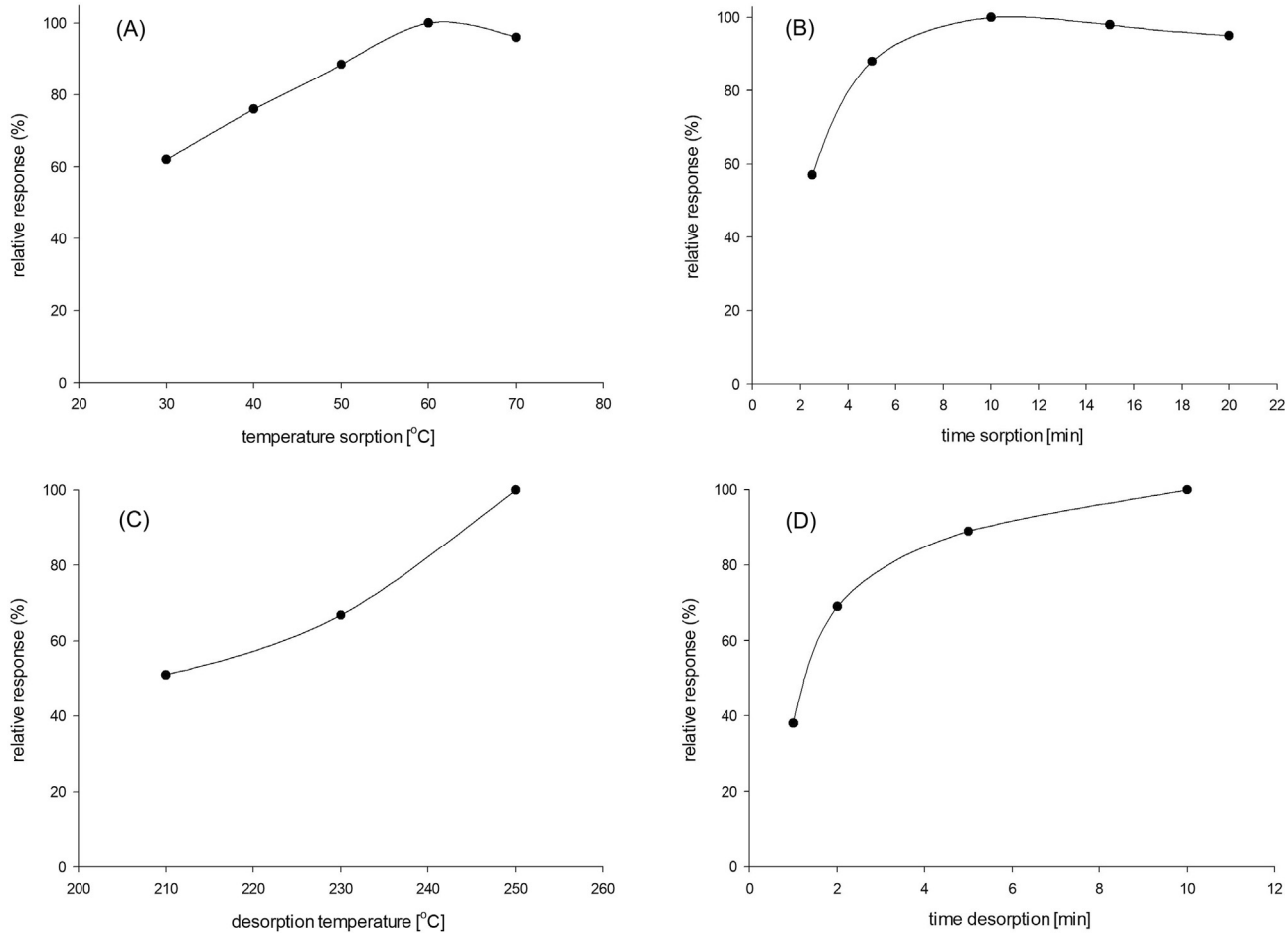

Fig. 3. Optimization of sorption/desorption – conditions: (A) optimization temperature sorption: 30 min; (B) optimization time sorption: 70 °C; (C) optimization temperature desorption: 2 min; (D) optimization time desorption: 250 °C.

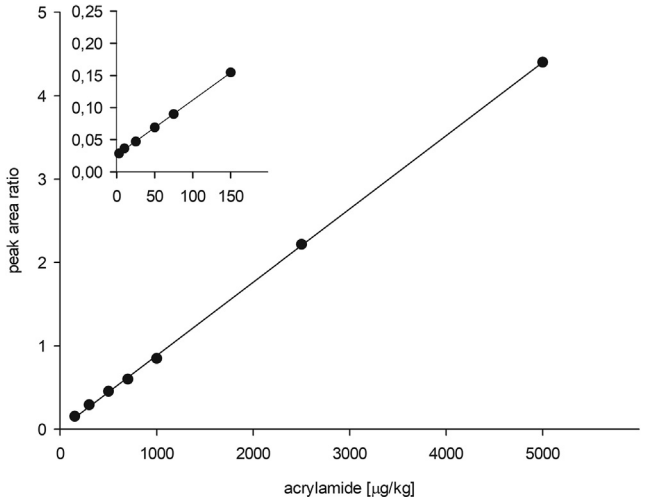

Fig. 4. Calibration curves for acrylamide using d_3-acrylamide internal standard (described in Section 3.4 Analytical method validation).

species and the way they were roasted, because they came from different countries as: Germany, France, the Netherlands and Poland. The level of acrylamide in coffee was determined in non-spiked grains and spiked 100, 500 and 2500 µg/kg. The values of the HORRAT coefficient between 1.00 and 1.20 demonstrates that described method was characterized by a good precision.

The combined standard uncertainty for determining of acrylamide in roasted coffee was calculated using the law of propagation. The budget included the relative standard uncertainty of the determination of standard concentration for preparation of the calibration curve, and the relative standard uncertainty for preparation of roasted coffee sample. The calculations included the purity of the applied standards and such operations as pipetting, weighing. The budget also included standard deviations related to the repeatability of chromatographic measurements. The expanded uncertainty was estimated for a level of confidence (p) equal to 95%, corresponding to a coverage factor of k = 2, by multiplying the combined standard uncertainty. The relative

Table 1
Selected parameters of HS-SPME/GC-MS method for the acrylamide determination in the green unroasted coffee spiked acrylamide.

Analytical procedure parameters	Criteria	HS-SPME/GC-MS
limit of determination (LOD) [µg/kg]	S/N ≥ 3	1
limit of quantification (LOQ) [µg/kg]	S/N ≥ 10	3
low concentration of acrylamide:		
working range of the method [µg/kg]	–	3 ÷ 150 (6 points)
linearity	r ≥ 0.995	r = 0.99971
slope of the line	–	$8.53 \cdot 10^{-4}$
y-intercept	–	$2.65 \cdot 10^{-2}$
high concentration of acrylamide:		
working range of the method [µg/kg]	–	150 ÷ 5000 (7 points)
linearity	r ≥ 0.995	r = 0.99980
slope of the line	–	$8.79 \cdot 10^{-4}$
y-intercept	–	$7.37 \cdot 10^{-3}$
repeatability – RSD [%]	RSD < 10%	2.6% (n = 3)
intermediate precision – RSD [%]	RSD < 10%	9.4% (n = 6)

Table 2
Selected parameters of HS-SPME/GC-MS method for acrylamide determination in the roasted coffee.

Analytical procedure parameters		Sample*			
		1	2	3	4
mean [μg/kg]		212.5	77.7	145.4	189.7
range of results [μg/kg]		209 ÷ 219	75 ÷ 79	144 ÷ 149	187 ÷ 195
expanded uncertainty (k = 2, p < 95) [%]		± 3.0	± 3.5	± 2.7	± 2.8
HORRAT coefficient		1.20	1.16	1.00	1.09
recovery at different levels of acrylamide addition [%] (RSD, %)	100 [μg/kg]	105 (5.3)	104 (4.9)	102 (4.6)	103 (4.5)
	500 [μg/kg]	104 (3.6)	101 (4.1)	104 (4.3)	104 (3.2)
	2500 [μg/kg]	102 (3.4)	99 (4.2)	101 (4.1)	101 (3.2)

* Sample: 1 Arabica – strongly roasting; 2 Arabica – middle roasting; 3 Robusta – middle roasting; 4 Robusta, middle roasting.

value of expanded uncertainty of the results of determining the acrylamide in coffee was between ± 2.7–3.5% and is provided in Table 2.

Based on the results given in the Tables 1 and 2, it can be concluded that the method is selective, has good precision, repeatability and accuracy.

4. Conclusion

The method validation confirms the effectiveness of the HS-SPME procedure for isolating acrylamide from coffee beans and their determination by GC-MS. The LOQ applying the described method of determination of acrylamide in coffee beans. Estimated precision was 3 μg/kg what is in line with the criteria defined by EU Commission Recommendations (Commission Recommendation (EU) 2013/647, 2013), on the monitoring of acrylamide levels in food. The described method demonstrates satisfactory repeatability (RSD = 2.6%) and intermediate precision (average RSD = 9.4%) for unroasted coffee beans spiked with acrylamide and good accuracy for roasted coffee (recovery 99–105%).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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