Rapid and reliable QuEChERS-based LC-MS/MS method for determination of acrylamide in potato chips and roasted coffee

S Stefanović¹, V Đorđević¹ and V Jelušić²

¹ Institute of Meat Hygiene and Technology, Kacanskog 13, Belgrade, Serbia
² Veterinary Office of Bosnia and Herzegovina, Maršala Tita 9a/II, Sarajevo, Bosnia and Herzegovina

E-mail: srdjan.stefanovic@inmes.rs

Abstract. The aim of this paper is to verify the performance characteristics and fitness for purpose of rapid and simple QuEChERS-based LC-MS/MS method for determination of acrylamide in potato chips and coffee. LC-MS/MS is by far the most suitable analytical technique for acrylamide measurements given its inherent sensitivity and selectivity, as well as capability of analyzing underivatized molecule. Acrylamide in roasted coffee and potato chips was extracted with water:acetonitrile mixture using NaCl and MgSO₄. Cleanup was carried out with MgSO₄ and PSA. Obtained results were satisfactory. Recoveries were in the range of 85-112%, interlaboratory reproducibility (Cv) was 5.8-7.6% and linearity (R²) was in the range of 0.995-0.999. LoQ was 35 μg kg⁻¹ for coffee and 20 μg kg⁻¹ for potato chips. Performance characteristic of the method are compliant with criteria for analytical methods validation. Presented method for quantitative determination of acrylamide in roasted coffee and potato chips is fit for purposes of self-control in food industry as well as regulatory controls carried out by the governmental agencies.

1. Introduction
Acrylamide (prop-2-enamide, 2-propene amide, CAS No. 79-06-1) is a compound that attracts much attention of the scientific, regulatory and food business operators’ communities for the past decade, due to its almost ubiquitous presence in some thermally processed foods, as well as its harmful properties to humans. International Agency for Research of Cancer (IARC) classified acrylamide as “probably carcinogenic to humans”, belonging to Group 2A [1]. Besides carcinogenicity, studies have attributed some other harmful properties to acrylamide, such is neurotoxicity [2]. Although the studies from available literature have not yet fully explained all possible formation mechanisms of acrylamide, it is well known that one way of its formation is based on Maillard reaction between amino acid asparagine and carbonyl-group compounds, especially reducing sugars e.g. glucose or fructose, at the temperatures higher than 120°C [3, 4]. Therefore, occurrence of acrylamide is high in carbohydrates-rich, thermally treated foods (potato chips, French fries biscuits, snacks of large varieties, roasted coffee, cereals, etc). On the other hand, Maillard reaction is responsible for desirable sensory properties of processed foods (dark yellow to brown color, crusty surface and rich taste).
However, at the same time, these fried or baked surfaces contain the highest quantities of acrylamide [5].

Since acrylamide presence in processed foods clearly poses a health risk to consumers, due to harmful properties of the molecule itself, as well as considering the abundance of acrylamide-containing product in our nutrition, European Commission initiated monitoring of acrylamide levels in processed foods in 2007 [6]. This survey ended in 2009 followed by another monitoring cycle [7]. Based on its data, in 2012 European Food Safety Agency (EFSA) concluded that no significant reduction of acrylamide levels is observed, except in food for infants and some other categories [8]. The European Commission published in 2013 its second Recommendation on “indicative acrylamide values based on monitoring data from 2007-12” [9] setting the maximum recommended levels e.g. for potato chips to 1000 µg kg\(^{-1}\) and for roasted coffee to 450 µg kg\(^{-1}\). Along with recommended levels, EC has promoted “toolbox” compiled by the organization “FoodDrinkEurope” with the aim to help food business operators to reduce acrylamide levels in their products [9].

From the analytical perspective, acrylamide detection and measurement is not without hindrances, regardless of relatively long period of availability of analytical methods. Historically, one of the first protocols describes analysis of acrylamide in drinking water after derivatization (bromination with 2,3-dibromopropionamide), and GC separation using ECD or MS detection [10]. Although this method can be applied to complex matrices such as various foodstuffs [11], procedure is time-consuming and with limited success in respect to reproducibility. HPLC methods with UV detection are also available, however, lack of chromophore and general limited selectivity and sensitivity of UV detectors make detection of low levels of acrylamide difficult and with questionable reproducibility. Liquid chromatography coupled with tandem-mass spectrometry (LC-MS/MS) is by far the most suitable analytical technique for acrylamide measurements given its inherent sensitivity and selectivity, as well as capability of analyzing underivatized molecule. Since molecular ion of acrylamide is of low weight (m/z = 72 for protonated molecule), it is not specific enough for an unambiguous spectral determination using full scan. Therefore, multiple reaction monitoring (MRM) mode of operation with triple-quadrupole instruments is the technique of choice for accurate and precise measurements of acrylamide content.

Another difficulty encountered in acrylamide analysis is practical impossibility to obtain true blank sample. The need for matrix-matched calibration, especially in mass spectrometry (due to often severe matrix-related effects), implies use of fortified blanks and blank samples in order to properly quantify the compound of interest. However, virtually every thermally processed matrix contains some levels of acrylamide. Furthermore, application of high temperatures during analytical process (e.g. Soxlet extraction or GC injection) can also contribute to the formation of additional acrylamide levels, if precursors for Maillard reaction are present in the sample [2].

The aim of this paper is to verify the performance characteristics and fitness for purpose of rapid and simple QuEChERS-based LC-MS/MS method for determination of acrylamide in two food commodities that are likely to contain significant quantities of this molecule and are, at the same time, rather abundant in everyday nutrition - potato chips and coffee. The applied analytical method is slightly modified LC-MS/MS protocol presented by Mastovska and Lehotay [2]. QuEChERS (Quick Easy Cheap Effective Rugged Safe) is an extraction and cleanup method which gained much popularity in analytical community in the past decade, due to significant benefits in solvents reduction, costs, turnaround time and efficiency of extraction. Chemically, it is a form of dispersive solid phase extraction and cleanup protocol that provides satisfactory to excellent recoveries and adequate interferences removal. It is developed by the US Department of Agriculture for the purpose of multiresidual pesticide analysis [12]. However, it can be successfully applied to other analytes, including acrylamide. Although it is not suitable for ultra-trace (sub-ppb) levels, recommended maximum levels for acrylamide are quite within reach of its capabilities.
2. Materials and Methods
Samples of potato chips (n=6) and roasted grind coffee (n=6) were purchased from local supermarkets. Analytical standard of acrylamide (99%) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile, n-hexane and water of HPLC purity were acquired from the same manufacturer. QuEChERS extraction kit (1g NaCl + 4g MgSO₄ in 50 mL extraction tube; 50mg PSA + 150mg anhydrous MgSO₄ in dispersive 2mL SPE tube) were obtained from Agilent (Agilent Technologies CA, USA).

2.1. Sample preparation - potato chips
One and a half gram of previously grind potato chips was weighted into 50 mL QuEChERS tube (1g NaCl + 4g MgSO₄). Standard of acrylamide was added at this point in QC samples (matrix-matched standards and control spike). HPLC-grade n-hexane (7mL) was added and the tube was vortexed for 1 min. HPLC-grade water and acetonitrile (10 mL each) were added to the tube and shaken vigorously for 1 min. This mixture was centrifuged at 4500g for 5 min. Hexane layer (upper) was discarded using Pasteur pipette and 1 mL of water:acetonitrile layer (lower) was transferred into 2mL dispersive SPE tube containing 50mg PSA and 150mg of anhydrous MgSO₄. Tubes were vortexed for 1 min and centrifuged at 9000g for 5 min. Supernatant (1 mL) was carefully withdrawn using automatic pipette in order not to disturb precipitate and transferred into HPLC vial.

2.2. Sample preparation - roasted coffee
Coffee samples (2g) were transferred into an empty tube and 10 mL of boiling water was added. Tube was closed and shaken vigorously for one minute. Entire content was transferred to QuEChERS extraction tube. The other steps were identical as in potato chips preparation except for the defattening step with n-hexane which was omitted. During the method development, it was concluded that interferences from fat were much less intense, comparing to potato chips while the peak shape and baseline noise were satisfactory. Therefore, in the interest of simplicity and time-saving, defattening step was skipped.

2.3. LC-MS/MS analysis
LC-MS/MS system consisted of Shimadzu (Shimadzu Corporation, Kyoto, Japan) components: two LC-30AD UPLC pumps connected in binary gradient mode, DGU-20A degassing unit, SIL-30AC autosampler, CTO-20AC column oven, CBM-20A system controller and LCMS 8040 triple-quadrupole mass spectrometer. Merck (Darmstadt, Germany) Purospher® STAR RP-18 encapped column (50x2.1mm, 3µm) was used for acrylamide separation from co-eluting compounds and was maintained at 50°C during analysis. Mobile phase consisted of 2% methanol and 1% acetonitrile in 0.1% formic acid(isocratic mode), flow rate was 300µL/min. Mass spectrometric determination of acrylamide was performed in electrospay (ESI) positive mode. Interface voltage was set to 4kV. Heat block, interface and desolvation line temperatures were 400°C, 350°C and 250°C respectively. Nebulizing gas (N₂) flow was 3 L/min while drying gas flow was maintained at 15 L/min. Detector was operating in multiple reaction monitoring (MRM) mode. Collision gas was Ar₂. Two product ions of acrylamide were monitored (m/z 72>55 and 72>27). Dwell time was set to 100 ms. Adequate collision energy, Q1 and Q3 pre bias values were determined using automatic method optimization procedure. LabSolutions software was responsible for data acquisition and processing.

Five-point calibration curve was constructed from pure standards corresponding to final sample concentrations of 0, 50, 100, 500 and 1000µg kg⁻¹. Samples of potato chips and coffee previously purchased from the supermarkets were analyzed prior to analysis, and specimens with lowest content of acrylamide were selected for blanks. Selected "blank" samples were fortified with acrylamide standard solution (c=1 ng µL⁻¹ in acetonitrile) up to the final concentrations in a sample of 100, 200, 500 1000 and 1500 µg kg⁻¹. Spiked samples prepared in such a way were used for construction of matrix-matched calibration curve. Blank subtraction and external standardization methods were used for quantification of acrylamide content.
3. Results
Described method showed good performance characteristics for both matrices. Degree of interferences removal during extraction and cleanup stages of sample preparation was sufficient for reliable analyte identification and subsequent quantification. Figure 1 represents MRM chromatogram of total ion current and both transition products for acrylamide pure standard (0.2 ng on column). Retention time of acrylamide was 0.5 min due to short column (50 mm) and addition of 1% acetonitrile to the mobile phase. Initial run time was set to 4 minutes, however subsequent coelution with other compounds was not observed in both sample types. Separation of acrylamide was clear with narrow peak with slight tailing. This could be reduced with alteration of chromatographic parameters but runtime of the analysis would be longer.

Baseline noise was prominent in coffee samples at low levels (under 50 µg kg\(^{-1}\)), while this effect was not observed in potato chips samples. This could be explained by the fact that coffee is rather difficult matrix compared to potato chips with much more coeluting compounds. Omission of defattening step in coffee analysis was excluded as a reason for increased baseline noise, since the same noise was observed in samples subjected to defattening with n-hexane during method development. Even with increased noise, peak was sufficiently resolved and reproducible integration and quantitation could be achieved. Figures 2 and 3 show MRM chromatograms of total ion current and both transition products for acrylamide in coffee and potato chips respectively at concentrations of 100 µg kg\(^{-1}\).

Figure 1. Chromatogram of acrylamide standard solution (0.2 ng on column)

Figure 2. Chromatogram of 100 µg kg\(^{-1}\) of acrylamide in roasted coffee sample

Figure 3. Chromatogram of 100 µg kg\(^{-1}\) of acrylamide in potato chips sample
Coffee samples, as stated previously, were harder matrix to analyze comparing to potato chips. One of the main drawbacks was pronounced difficulty in obtaining credible blank samples. Six samples of commercially acquired roasted coffee were analyzed in preliminary investigations. All samples contained acrylamide in various concentrations. Unlike potato chips samples where it was possible to find a sample with low enough concentrations of acrylamide which could be considered as true blank, in the case of coffee samples, blank substraction method and standard addition were employed for reliable quantification.

With every batch of samples, control spike was injected after blank with the aim to assess recoveries of the method. Blank samples were fortified at 50 μg kg⁻¹ for coffee and 100 μg kg⁻¹ for potato chips. Obtained recoveries were in the range of 85-112% for coffee samples and 91-107% for potato chips samples.

Linearity of the method was satisfactory. Five-point calibration curve consisting of matrix-matched standard was constructed at the beginning of each batch. Coefficients of determination (R²) were in the range of 0.995-0.998 for coffee and 0.997-0.999 for potato chips.

Experimentally determined limit of quantification was 35μg kg⁻¹ for coffee and 20 μg kg⁻¹ for potato chips. Interlaboratory reproducibility (Cv%) at recommended indicative values from the European Commission (450 μg kg⁻¹ for roasted coffee and 1000 μg kg⁻¹ for potato chips) were 7.6% and 5.8% respectively.

Accuracy of the method was initially difficult to assess due to the lack of adequate certified reference material (CRM). Recovery could be a measure of accuracy when CRMs are not available, but practical impossibility to obtain true blank matrix further complicates this process. However, subsequent proficiency test for acrylamide in roasted coffee and z-score of -1 confirmed that applied method was adequate for accurate measurings.

One aspect of quality control was not applied in this method - internal standardization since d₃-acrylamide was not available in laboratory at the time of method development and validation. Even with this, presented method showed adequate performances in analysing roasted coffee and potato chips.

4. Conclusion
Presented method for quantitative determination of acrylamide in roasted coffee and potato chips is fit for purposes of self-control in food industry as well as regulatory controls carried out by the governmental agencies. Performance characteristics of the method are compliant with criteria for analytical methods validation. Low solvent consumption, lack of derivatization, overall low cost of analysis and very high sample throughput in both preparation and analysis steps are key benefits of applying such method in order to protect consumers from high exposure to acrylamide in food.

Acknowledgments
This work was supported by the Ministry of Science and Technological Development of the Republic of Serbia, grant III46009.

References
[1] IARC 1994 Monographs on the evaluation of carcinogenic risks to humans: Some industrial chemicals 60 389-433
[2] Mastovska K and Lehotay S J 2006 J. Agric. Food Chem.54 7001-8
[3] Claes W L, Vieseschouwer K de and Hendrick M E 2005 Trends Food Sci. Technol. 16 181-93
[4] Tareke E, Rydberg P, Karlsson P, Eriksson S and Tornquist M 2002 J. Agric. Food Chem. 35(6) 909-12
[5] EPA (Environmental protection Agency) 2010EPA/635/R-07009F URL https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0286tr.pdf
[6] EU (European Union) 2007 Commission Recommendation of 3 May 2007 on the monitoring of acrylamide levels in food 2007/331/EC OJ L 123 33-40
[7] EU (European Union) 2010 Commission Recommendation of 2 June 2010 on the monitoring of acrylamide levels in food 2010/307/EU OJ L 137 4-10
[8] EFSA (European Food Safety Agency) 2012 Scientific report of EFSA. Update on acrylamide levels in food from monitoring years 2007-2010 EFSA Journal 10 10 2938
[9] EU (European Union) 2013 Commission Recommendation of 8 November 2013 on investigations into the levels of acrylamide in food 2013/647/EU OJ L 301 15-17
[10] Andrawes F, Greenhouse S and Draney D 1987 J. Chromatogr. 399 269-75
[11] Castle L, Campos M and Gilbert J 1991 J. Sci. Food Agric. 54 549-55
[12] Anastassiades M, Lehotay S J, Stajnbaher D and Sheck F J 2003 J. AOAC Int. 86(22) 412-31