

## Method development and validation for acrylamide in cereals products by UPLC-MS/MS

S. Copeto\*, S. Jesus, I. Delgado and I. Coelho

*Department of Food and Nutrition, National Institute of Health Doutor Ricardo Jorge (INSA), IP, Avenida Padre Cruz, 1649-016, Lisbon, Portugal*

The Maillard reaction is responsible for the colour, flavour, and aroma of food products. However, it also has the undesirable effect of producing harmful substances, such as acrylamide (AA). This unsaturated amide is formed when starchy foods, particularly bread, potatoes, and coffee, undergo processing. The European Union has classified AA as a carcinogen (Category 1B), mutagen (Category 1B), and reproductive toxicant (Category 2). This study aims to validate a method for determining AA in cereal products, as well as to investigate its levels in different cereal products.

Ultra-efficiency liquid chromatography coupled to a mass detector with ionization mode, ESI (+), was used to detect and quantify AA. The extraction method consists of adding AA-d3 as an internal standard, extraction with water, defatting with hexane and cleanup with SAX solid-phase extraction. Method performance and figures of merit were validated using a certified reference material (ERM-BD272, Crispbread) and proficiency tests. Several parameters were evaluated during method development and validation, including linearity, the limit of detection (LOD), the limit of quantification (LOQ), accuracy, precision and uncertainty. The LOD and LOQ were 0.19 µg/kg and 0.47 µg/kg, respectively, which comply with Commission Regulation (EU) 2017/2158. The resulting calibration curve exhibited linearity within two working ranges (0.5-10 µg/kg and 10-150 µg/kg) with an R-squared value higher than 0.995. The proposed method demonstrated good recovery rates (77%-106%) and precision. These validation results, combined with the proficiency test of FAPAS Crispbread (Z-score: 0.69) and Biscuit (Z-score: -0.93) and ERM-BD272 results, demonstrate that this quantitative method for AA determination in cereal products is fit for purpose.

Following the method was applied to market available Cereal-based products such as Maria wafer (n=6), wheat bread (n=6), cornflakes (n=6), and baby cookies (n=6), collected from the local market in Lisbon, Portugal. AA results were expressed in µg/kg.

The validation procedures evidenced the suitability of the analytical method, which was effective in achieving an accurate determination of AA in cereals products.

**Keywords:** acrylamide, solid-phase extraction, cereal products, method validation occurrence

\* E-mail: [sandra.copeto@insa.min-saude.pt](mailto:sandra.copeto@insa.min-saude.pt)