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Robust Quantification of Acrylamide in Food using Gas Chromatography-Single Quadrupole Mass Spectrometry

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GOAL

To demonstrate a simple, cost-effective analytical solution for the routine determination of low level acrylamide in food and coffee samples, from sample extraction to detection and quantification, using a Thermo Scientific™ ISQ™ 7000 GC-MS system coupled with a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph and Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software.

INTRODUCTION

Acrylamide (2-propeneamide) is a chemical that has been found in certain cooked foods e.g., fried and baked starchy foods such as potato crisps and chips, roasted coffee, breads, peanuts, and cigarette smoke.[1,2] In baked and fried foods, acrylamide is formed as a by-product of the Maillard reaction, occurring between asparagine and reducing sugars (fructose, glucose etc.) or reactive carbonyls at temperatures above 120 °C.[1,3,4,5]

Acrylamide is highly toxic, can cause neurotoxicity, genotoxicity and reproductive harm, and is a likely human carcinogen.[6] The Food Standards Agency (FSA) regulations 2017/2158 have detailed legislation concerning acrylamide levels in food, guidance for food business operators and benchmark levels of acrylamide in different food categories.[7]

Current sample preparation and analytical technologies used for the analysis of acrylamide, involve extraction methods such as Soxhlet extraction, liquid-liquid extraction, and solid phase extraction (SPE), which are time consuming and require large amounts of organic solvents, followed by using either liquid chromatography/tandem mass spectrometry (LC-MS/MS), or gas chromatography (GC) coupled to an electron capture detector (ECD), flame ionization detection (FID), or mass spectrometry (MS). Due to its high-water solubility, aqueous extraction followed by LC-MS/MS has emerged as the main method for the analysis of acrylamide from food matrices. However, water will also extract high molecular weight compounds, including proteins, and often requires time-consuming sample clean-up.[8] Current GC-MS methods mainly involve derivatization via bromination,[9] which is labor intensive, and may cause breakdown of the brominated acrylamide in the GC injector or column at high temperatures.

This work aims to overcome the analytical challenges of current methods applied for acrylamide analysis in food by considering a cost-effective, robust, and selective approach that uses acetonitrile as the extraction solvent and derivatization using silylation, followed by GC-MS for the analysis of food and coffee samples.

MATERIALS AND METHODS

Sample preparation

Various food and coffee samples were purchased locally for targeted quantitative analysis of acrylamide, using splitless injection. The analytical workflow for the analysis of acrylamide is illustrated in Figure 1.

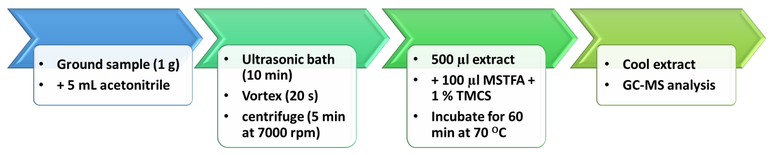

Figure 1. Acrylamide analytical workflow.

To assess acrylamide linearity, working calibration solvent standards were prepared in acetonitrile, subjected to the derivatization steps described previously (ranging from 1 ppb to 1000 ppb, equivalent to 5-5000 µg/kg in the sample).

Instrument and method setup

An ISQ 7000 GC-MS system was used in all experiments, configured with the vacuum probe interlock (VPI) and the ExtractaBrite source, operated in selected ion monitoring mode (SIM) using electron ionization (EI) coupled with a TRACE 1310 Gas Chromatograph equipped with a Thermo Scientific™ Instant Connect split/splitless (SSL) injector, and configured with a Thermo Scientific™ TriPlus™ RSH™ autosampler. Additional details on instrument parameters are listed in Tables 1 and 2.

Table 1. GC and injector conditions.

TRACE 1310 GC system parameters				
Liner:	Splitless liner, single taper, 4.0 mm x 6.5 mm x 78.5 mm			
Inlet temperature (°C):	250			
Carrier gas, mL/min, mode:	He, 1.2, constant flow			
Inlet module and mode:	SSL, splitless			
Split flow (mL/min):	100			
Splitless time (min):	2			
Splitless time (min):	2			
Septum purge flow (mL/min):	5			
Column:	Thermo Scientific™ TraceGOLD TG-WaxMS 30 m x 0.25 mm I.D x 0.25 µm			
Injection volume (µL):	1.0			
Septum purge flow (mL/min):	5			
Oven temperature program:	RT (min)	Rate (°C / min)	Target temperature (°C)	Hold time (min)
Initial	0	-	50	2.0
Stage 1	2.0	3	100	0.0
Final	18.7	25	250	5.0
Run Time	30	-	-	-

Table 2. Mass spectrometer conditions

MS conditions:	
Transfer line (°C):	250
Ionization mode:	EI (ExtractaBrite)
Ion source (°C):	250
Electron energy (eV):	70
Acquisition mode:	Timed selected ion monitoring (t-SIM)
SIM ions:	*m/z* 128 (quantification ion) and *m/z* 85 (confirming ion)

Data Processing

Data were acquired, processed, and reported using Chromeleon CDS software, version 7.2. Chromeleon CDS software allows the analyst to set up acquisition, processing, and reporting methods with easy data reviewing and flexible data reporting.

RESULTS

The object of this study was to evaluate the utility of a simplified approach that uses GC-MS to analyze acrylamide in food. For this, MSTFA was employed to derivatize acrylamide. In depth investigation on the derivatization parameters, including derivatization volume, temperature and time was performed. The analytical method was tested by considering various analytical parameters, including selected ion monitoring (SIM) conditions, chromatographic resolution, linearity, sensitivity, repeatability and robustness in matrix, and selectivity.

Data Processing

Gaussian chromatographic peak shapes and narrow peak widths are vital in determining accurate peak areas, improved sensitivity (lower detection limits), and in turn, precise concentrations of the target analytes. Using the GC conditions described in Table 1, the peak shapes obtained are shown in the extracted ion chromatograms (EIC, *m/z* 128) for acrylamide in solvent standards, samples containing incurred residues and spiked samples (Figures 2A, 2B and 2C accordingly).

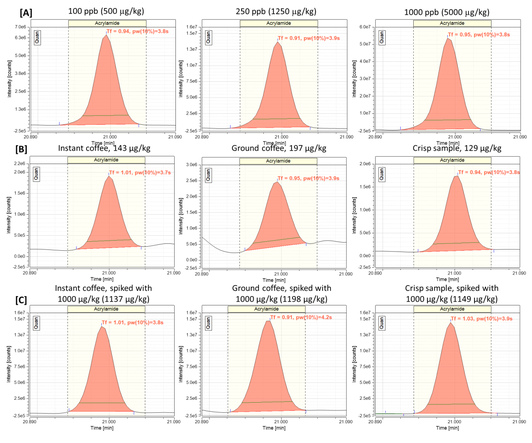

Figure 2. Example of chromatographic separation of acrylamide in A: derivatized calibration working standards at 100, 250 ppb and 1000 ppb, B: derivatized samples, instant coffee, ground coffee and crisps, and C: spiked samples, instant coffee, ground coffee and crisps. Annotated with tailing factor (Tf) and peak width, measured at 10% (green line). Samples and spiked sample results quoted using standard addition calibration.

Peak asymmetry values for acrylamide, with tailing factors (T_f) between 0.91-1.01 (indicating almost perfect Gaussian peak shapes), and narrow peak widths of ~4 s were observed, measured at 10% (illustrated in Figure 2).

Linearity of response

External standard calibration

To assess linearity, 8 calibration levels quantified using an external solvent standard calibration using a 1/x weighting factor. An example calibration curve for acrylamide is shown in Figure 3.

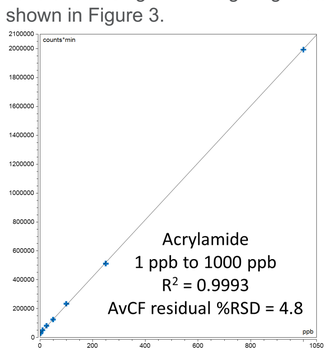

Figure 3. Example calibration curve for acrylamide, illustrating the excellent linearity obtained, over 8 calibration levels ranging from 1 to 1000 ppb (equivalent to 5-5000 µg/kg in food samples). Annotated with coefficient of determination (R^2) and the average calibration factor (AvCF) (as %RSD).

Standard addition calibration

Standard addition calibration was also used, to compensate for matrix effects. Crisps, instant coffee and ground coffee samples unspiked, and spiked at 1000 µg/kg and 2000 µg/kg (3 replicates at each level) were quantified, using a 1/x weighting factor. Excellent linearity was demonstrated for acrylamide, with R^2 value of ≥ 0.9987 and AvCF %RSD of ≤ 4.0 achieved for crisps, instant coffee and ground coffee standard addition calibration curves, see Figure 4, where both the coefficient of determination (R^2) and the residual %RSD are annotated.

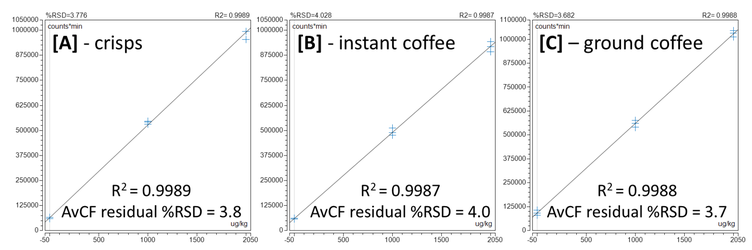

Figure 4. Standard addition calibration curve for A: crisps, B: instant coffee, and C: ground coffee, unspiked, and spiked at 2 levels (1000 µg/kg and 2000 µg/kg), 3 replicates at each level. Annotated with coefficient of determination (R^2) and the average calibration factor (AvCF) (as %RSD).

Peak area repeatability and robustness in matrix

Repeatability and robustness of acrylamide responses in matrix were assessed by carrying out repeated injections (n=16) of a QC ground coffee sample, spiked with 200 ppb acrylamide (equivalent to 1000 µg/kg) prior to extraction, as part of a 99 injections analytical sequence, containing derivatized blanks, calibration standards, crisp, instant coffee and ground coffee samples. Three QC injections were mid sequence (lines 46-48), with the additional 13 injections analysed near the end of the sequence (lines 79-92). Excellent repeatability is illustrated in Figure 5, with peak area %RSD of 2.9 for the acrylamide absolute peak area for all 16 injections, and robustness highlighted with peak area %RSD of 1.3 comparing the spiked samples injection mid sequence to those injected at the end of the analytical sequence. No inlet, column, MS maintenance, or MS tuning were performed over the injection sequence.

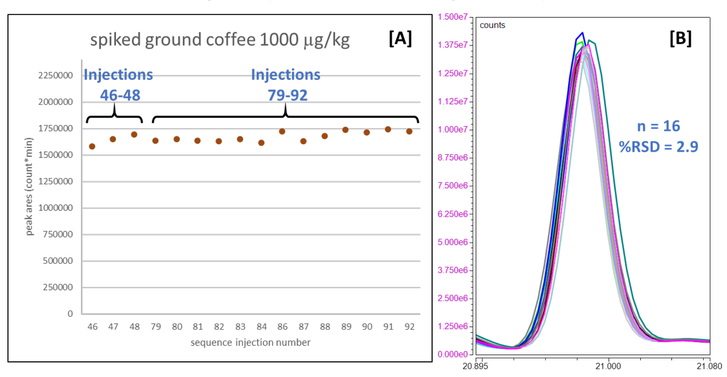

Figure 5. A: robustness data shown as consistent peak area counts for acrylamide determined in QC ground coffee samples spiked at 200 ppb (equivalent to 1000 µg/kg), analysed mid (inj. No. 46-48), and end (inj. No. 79-92) of a 99 injections analytical sequence, containing derivatized blanks, calibration standards, crisp, instant coffee and ground coffee samples. B: overlaid EIC (*m/z* 128) of the QC ground coffee sample (n=16 injections) analysed across the whole analytical sequence. For all QC ground coffee samples containing acrylamide at the 200 ppb level across the analytical sequence of 99 injections the calculated %RSD absolute peak area counts was 2.9.

Selectivity in matrix

By using MSTFA as derivatization reagent, sensitivity and selectivity for the analysis of acrylamide is enhanced, (when compared to un-derivatized). Using acetonitrile instead of water as the extraction solvent, avoids the extraction of proteins and other high molecular weight compounds that could interfere chromatographically and compete for silylation reagent. Derivatized acrylamide, compared to the free acrylamide, has both greater chemical and thermal stability, which makes it more applicable to GC-MS analysis.

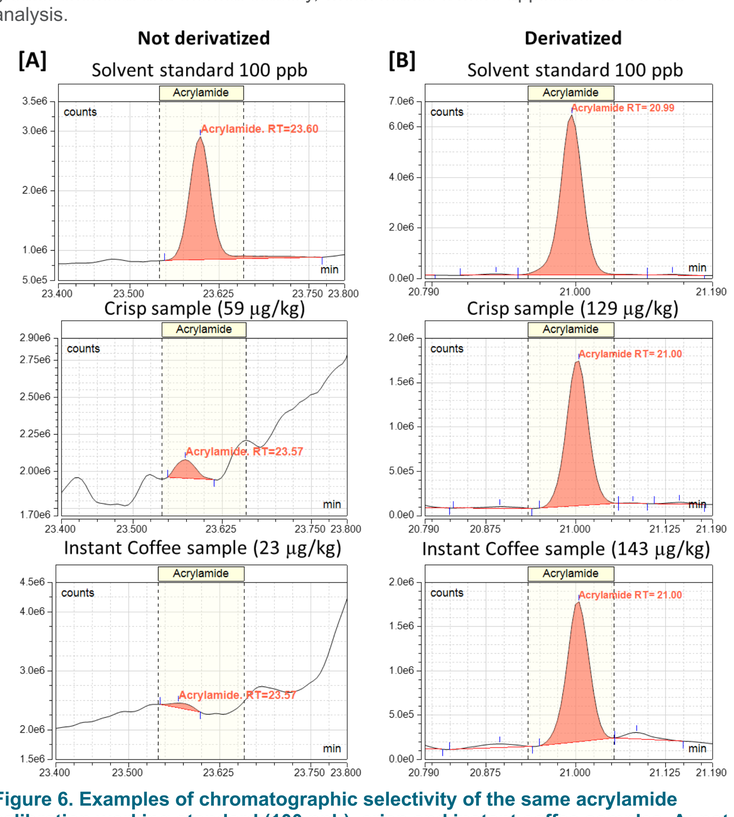

Figure 6. Examples of chromatographic selectivity of the same acrylamide calibration working standard (100 ppb), crisp and instant coffee samples, A: not derivatized (*m/z* 55), and B: derivatized with MSTFA + 1% TMCS (*m/z* 128). Sample results quoted using standard addition calibration.

Compared to detection of free acrylamide (without derivatization), co-extracts of low *m/z* ions, which can interfere with acrylamide, which in matrix can markedly affect the detection limits, lead to erroneous detection and inaccurate results. This is demonstrated in Figure 6, which illustrates the chromatographic separation and example results achieved for the same samples and standards, but with and without derivatization. For the non-derivatized analysis, the same calibration solvent standards were analyzed, acquiring *m/z* 55 (quantification ion) and *m/z* 71 (confirming ion) and resulting in linearity with R^2=0.9989, and residual %RSD of 6.0. Figure 6A, shows that for the same sample extract, the non-derivatized chromatogram resulted in closely eluting peaks, which makes the integration and associated result achieved questionable. For the derivatized samples there was a significant increase in signal response and improvements in selectivity.

Quantification of acrylamide in food samples

Samples of crisps and coffee (instant and ground) were prepared and analyzed in triplicate using the derivatization protocol. Samples were analyzed before spiking, to determine the acrylamide content, and spiked at two levels (1000 and 2000 µg/kg) to assess recovery and method precision. Acrylamide quantification was performed using a standard addition calibration for each matrix, which eliminated the need for an expensive ^{13}C labelled internal standard. A summary of results for crisps, instant and ground coffee samples are shown in Figure 7.

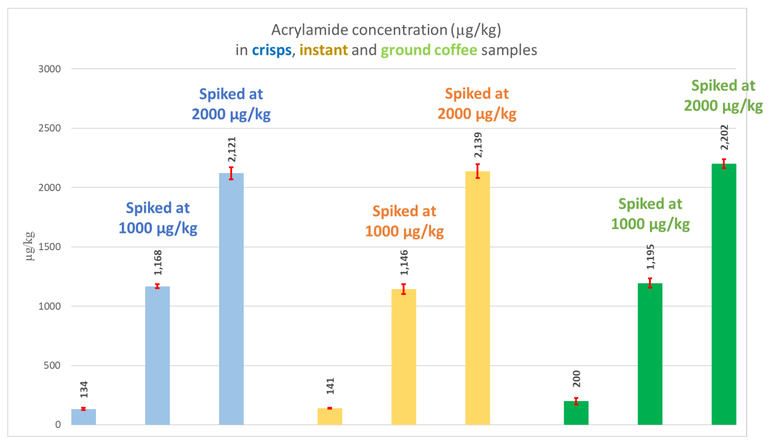

Figure 7. Average concentration of acrylamide (n=3) using standard addition calibration determined for unspiked and spiked (1000 and 2000 µg/kg) crisp, and instant and ground coffee samples, showing consistency at low and high levels. Standard deviation calculated from the three replicates is annotate, demonstrating the repeatability of the method.

CONCLUSIONS

The results obtained clearly demonstrate that the ISQ 7000 GC-MS system with a TRACE 1310 Gas Chromatograph, in combination with the TriPlus RSH autosampler and the Chromeleon CDS software, offers a viable alternative to laboratories that analyze low level contaminants such as acrylamide in food commodities. This statement is based on the following findings:

- Good chromatographic resolution with excellent peak asymmetry values (tailing factors between 0.91-1.01), and peak width (+10 %) ≤ 4 seconds were achieved.
- Compound linearity obtained for derivatized acrylamide over a calibration range of 1 to 1000 ppb, resulted in average coefficient of determination R^2 of 0.9993 and average residual %RSD of 4.8.
- Excellent linearity was also demonstrated using standard addition calibration for acrylamide, to compensated for matrix effects, samples unspiked, and spiked at 1000 µg/kg and 2000 µg/kg, with R^2 value of >= 0.9987 and average residual %RSD of <= 4.0 achieved for crisps, instant coffee and ground coffee samples.
- The sensitivity of the method, defined as the limit of identification (LOI), of 1 ppb (equivalent to 5 µg/kg in the analyzed samples) was achieved using the detailed method.
- Excellent repeatability was achieved for the analysis of spiked ground coffee samples, 1000 µg/kg (n=16) achieving a %RSD of 2.9.
- Robustness of acrylamide responses in matrix was assessed by analyzing spiked ground coffee samples injected mid sequence (n=3), with additional samples analyzed near the end of the sequence (n=13) with %RSD of 1.3 when comparing average peak areas of mid to late sequence injected spiked samples. In addition, no inlet, column, MS maintenance, or MS tuning were performed over the injection sequence.
- The analyses of food and coffee samples using the developed method, non-spiked and spiked, at two levels (1000 and 2000 µg/kg) was used to access recovery and method precision. Acrylamide quantification was performed using standard addition calibration, eliminating the need for an expensive ^{13}C labelled internal standard. The results illustrated consistency of results at low to high levels.
- Silylation of food and coffee samples extracted with acetonitrile, quantified in SIM mode, maximizes sensitivity and selectivity for the analysis of acrylamide. The enhanced chemical and thermal stability of the silylated product compared to non-derivatized acrylamide analysis, makes the analysis using silylation more applicable to GC-MS analysis.
- Chromeleon CDS software simplifies the workflow with user-friendly data acquisition and data processing, suitable for high-throughput analysis, with intuitive data reviewing and flexible data reporting.

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