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Simple and cost-effective determination of acrylamide in food products and coffee using gas chromatography-mass spectrometry

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Keywords

Acrylamide, gas chromatography, mass spectrometry, GC-MS, single quadrupole mass spectrometry, ISQ 7000, vacuum probe interlock, VPI, ExtractaBrite, silylation, derivatization, MSTFA

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, including fried and baked starchy foods, such as potato crisps (potato chips) and chips (French fries), 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) regulation 2017/2158 provides legislation concerning acrylamide levels in food, guidance for food business operators, and benchmark levels of acrylamide in different food categories.[7]

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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, which are costly to dispose of. They are followed by either liquid chromatography/tandem mass spectrometry (LC-MS/MS) or gas chromatography (GC) coupled to electron capture detection (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 determination of acrylamide in food matrices. Since water will also extract high molecular weight compounds, including proteins, a time-consuming sample clean-up is often required.[8] Current GC-MS methods mainly involve derivatization via bromination,[9] which is labor-intensive, and the brominated acrylamide may break down at high temperature in the GC injector or column.

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

Experimental

Sample preparation

Various food and coffee samples were purchased locally for targeted quantitative analysis of acrylamide, using splitless injection.

Five milliliters of acetonitrile were added to a ground sample (1 g). The sample was extracted in an ultrasonic bath (10 min) and vortexed (20 s). An aliquot (~1 mL) was centrifuged (5752 g for 5 min). Then, 500 µL of the supernatant was transferred to a crimp-top GC vial and 100 µL of the silylation reagent MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide) with 1% TMCS (2,2,2-trifluoro-*N*-methyl-N-(trimethylsilyl)-acetamide, chlorotrimethylsilane) as catalyst (P/N TS48915) was added. The solution was mixed and heated at 70°C for 60 min. After cooling naturally to room temperature, the sample extract was ready for analysis. The analytical workflow for the analysis of acrylamide is illustrated in Figure 1.

To assess acrylamide linearity and instrument performance, working calibration solvent standards were prepared in acetonitrile and subjected to the derivatization steps described previously (ranging from 1 ppb to 1000 ppb, equivalent to 5–5000 µg/kg in the sample). Standard addition calibrations were used for quantification, samples unspiked and spiked at 1000 µg/kg and 2000 µg/kg, and subjected to derivatization.

Instrument and method setup

An ISQ 7000 GC-MS system was used in all experiments. The MS was configured with the vacuum probe interlock (VPI) and the ExtractaBrite source, and was operated in timed selected ion monitoring (t-SIM) using electron ionization (EI). A TRACE 1310 Gas Chromatograph was equipped with a Thermo Scientific™ Instant Connect split/splitless (SSL) injector, and configured with a Thermo Scientific™ TriPlus™ RSH™ autosampler.

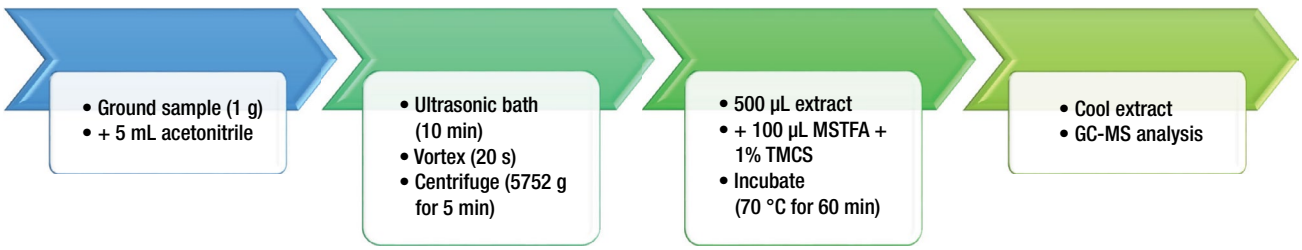

Figure 1. Acrylamide analytical workflow, highlighting the main steps of the process in which a low sample amount (1 g) is derivatized using silylation reagent prior to GC-MS analysis

Compound separation was achieved using a Thermo Scientific™ TraceGOLD™ TG-WaxMS 30 m × 0.25 mm i.d. × 0.25 µm film capillary column (P/N 26088-1420).

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 × 6.5 mm × 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
Septum purge flow (mL/min):	5
Column:	TraceGOLD TG-WaxMS 30 m × 0.25 mm i.d. × 0.25 µm
Injection volume (µL):	1.0

Oven temperature program

	RT (min)	Rate (°C/min)	Target Temp (°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

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 and discussion

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 of 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.

Chromatography

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

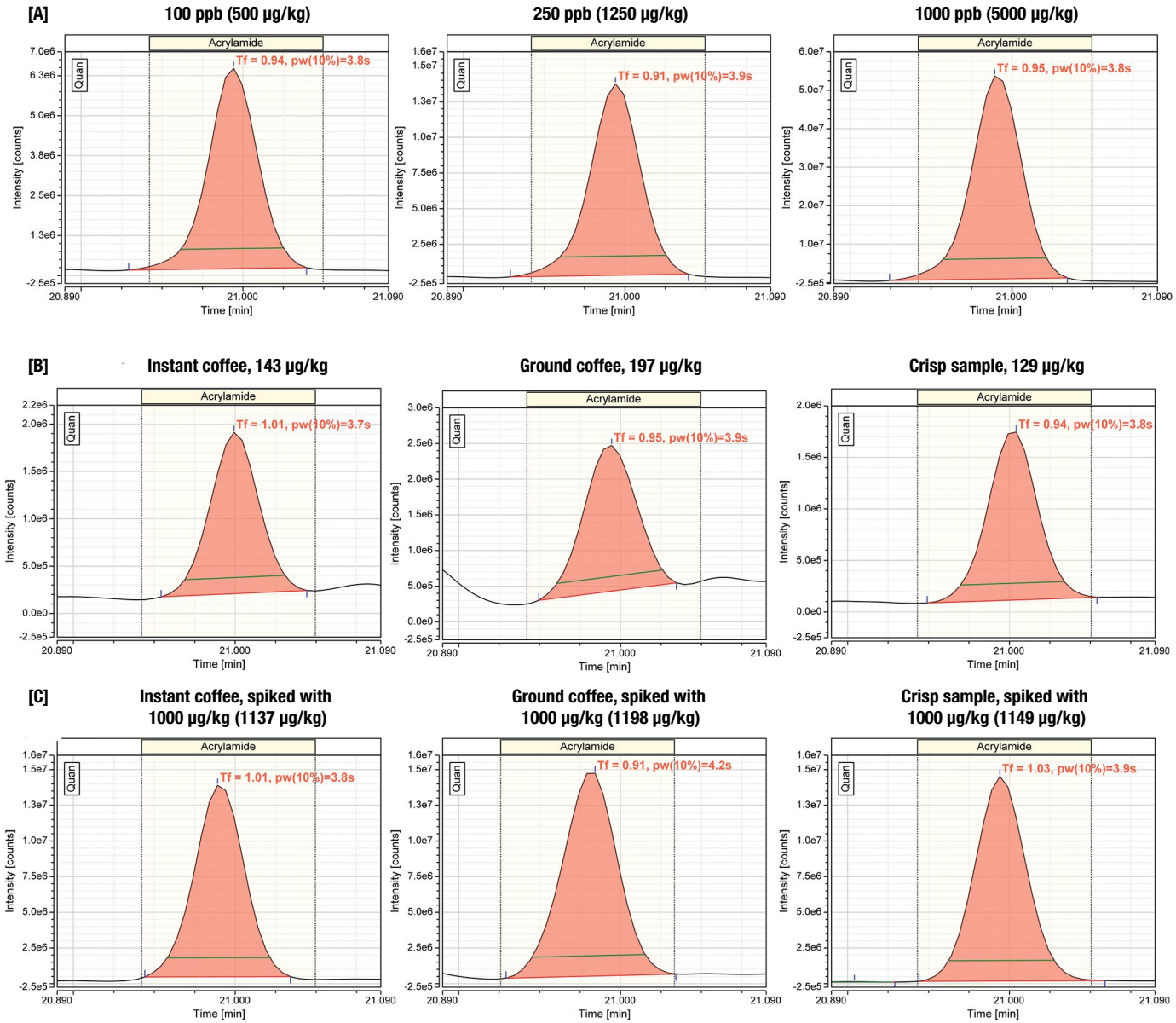

Figure 2. Example of chromatographic separation of acrylamide in A: derivatized calibration solvent standards at 100, 250, and 1000 ppb, B: derivatized samples, instant coffee and crisps, and C: spiked samples, instant coffee and ground coffee. Annotated with tailing factor (Tf) and peak width, measured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

Linearity of response

External standard calibration

Solvent standards were used to assess linearity and instrument performance. Linearity of external calibration was assessed using eight calibration levels (1 to 1000 ppb) prepared in solvent (equivalent to between 5 and 5000 µg/kg in the analyzed samples) using a 1/× weighting factor. Excellent linearity was demonstrated for acrylamide, with a coefficient of determination (R^2) of 0.9993 and an average residual %RSD (AvCF %RSD) of 4.8. An example calibration curve for acrylamide is shown in Figure 3 where both the R^2 value and the AvCF %RSD are annotated.

Standard addition calibration

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

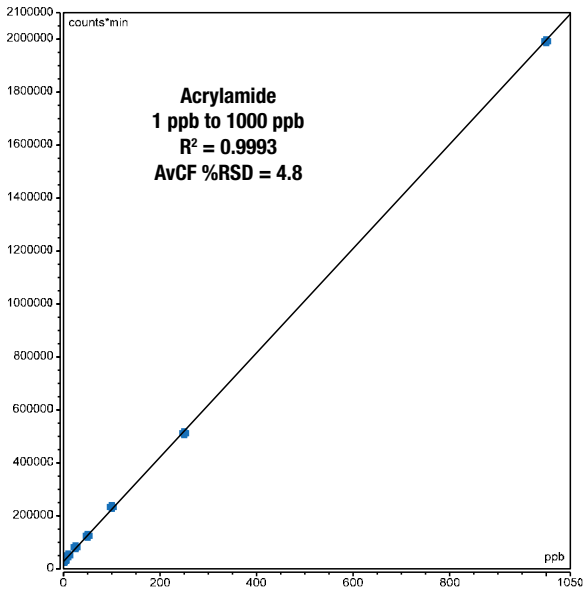

Figure 3. Example solvent calibration curve for acrylamide, illustrating the linearity obtained, over eight calibration levels ranging from 1 to 1000 ppb (equivalent to 5–5000 µg/kg in food samples). Annotated with coefficient of determination (R^2) and the average calibration factor (AvCF) (as %RSD).

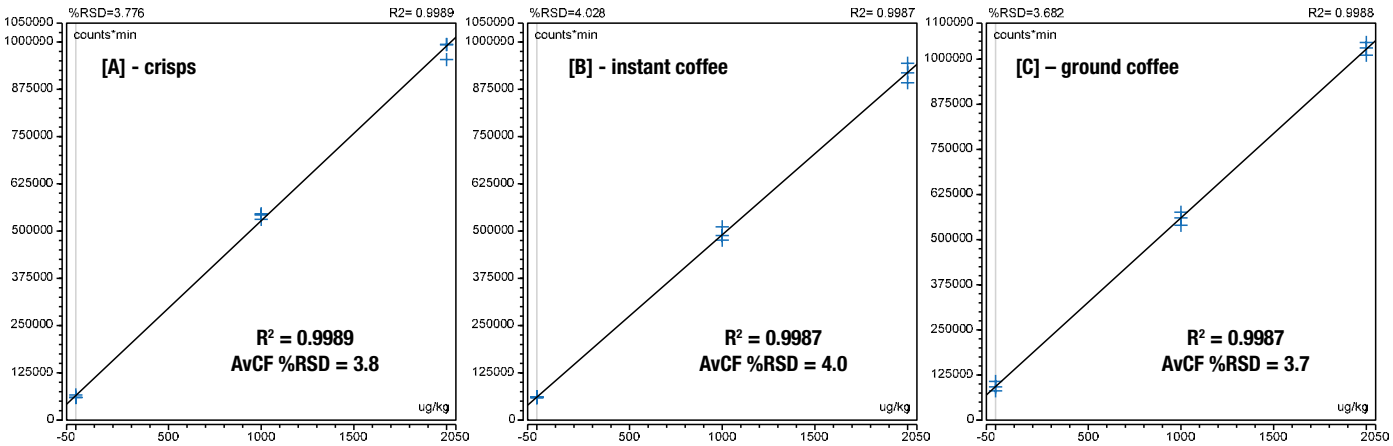

Figure 4. Standard addition calibration curve used for quantification for A: crisps, B: instant coffee, and C: ground coffee, unspiked, and spiked at two levels (1000 µg/kg and 2000 µg/kg), three replicates at each level. Annotated with coefficient of determination (R^2) and the average calibration factor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

Sensitivity

A limit of identification (LOI) of 1 ppb (equivalent to 5 µg/kg in the analyzed samples) was achieved using the detailed method (Figure 5). LOI is a measure of method sensitivity and was determined based on the criteria for identification of pesticide residue in food and feed (as outlined in the SANTE/11813/2017 guidelines) considering the lowest concentration of acrylamide solvent standard passing the criteria: Ion ratios within ±30% of the expected values calculated as an average across the calibration range 1 to 1000 ppb (equivalent to between 5 and 5000 µg/kg in the analyzed samples) and ion co-elution within ±0.01 minutes.

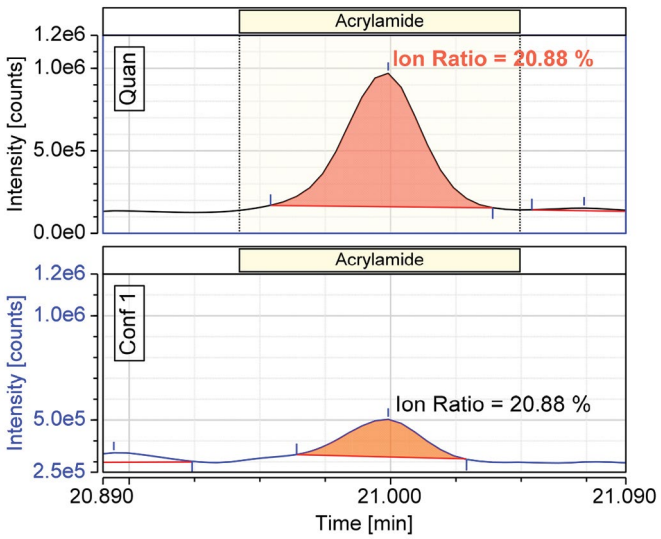

Figure 5. Extracted ion chromatograms for the quantification ion (*m/z* 128, upper) and the confirming ion (*m/z* 85, lower) at 1 ppb (LOI) for acrylamide. Ion ratio value achieved within ±30% of expected ion ratio (calculated as an average across the calibration range).

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-injection 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 analyzed near the end of the sequence (lines 79–92). Excellent repeatability is illustrated in Figure 6, with a 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.

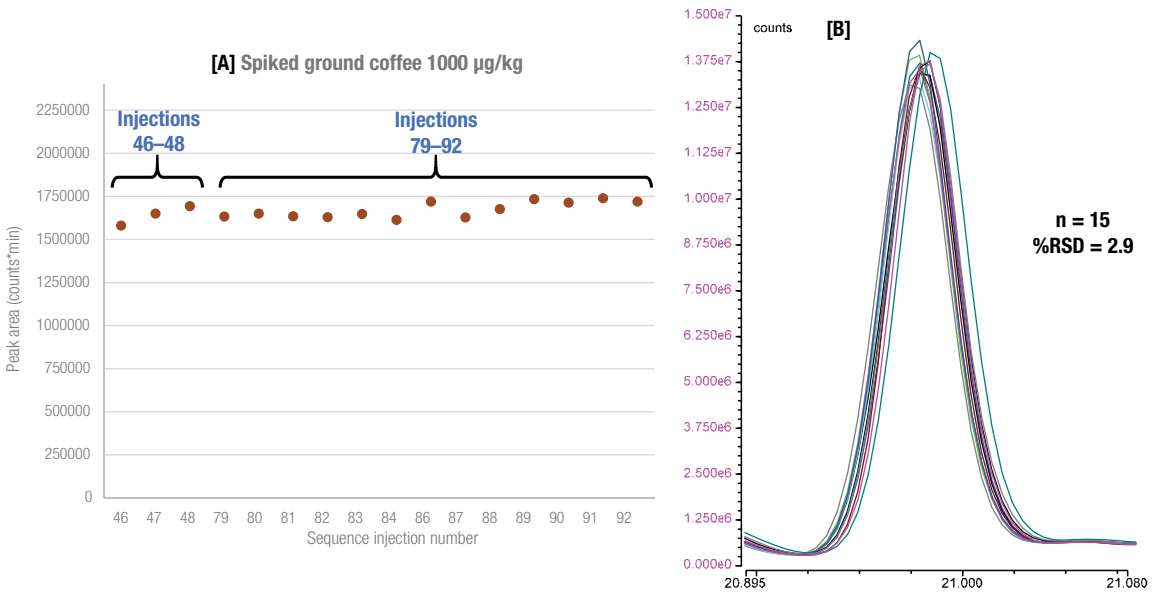

Figure 6. [A] Robustness data shown as consistent peak area counts for acrylamide determined in QC ground coffee samples spiked at 200 ppb (equivalent to 1000 µg/kg), analyzed mid (inj. no. 46–48), and end (inj. no. 79–92) of a 99-injection analytical sequence, containing derivatized blanks, calibration standards, crisp, instant coffee, and ground coffee samples. [B] overlaid EIC (*m/z* 128) of the QC ground coffee sample (n=16 injections) analyzed 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 the derivatization reagent, sensitivity and selectivity for the analysis of acrylamide is enhanced (when compared to non-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 the 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.

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 and lead to erroneous detection and inaccurate results. This is demonstrated in Figure 7, which illustrates the chromatographic separation and example results achieved for the same samples and standards, prepared as detailed, 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 7 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.

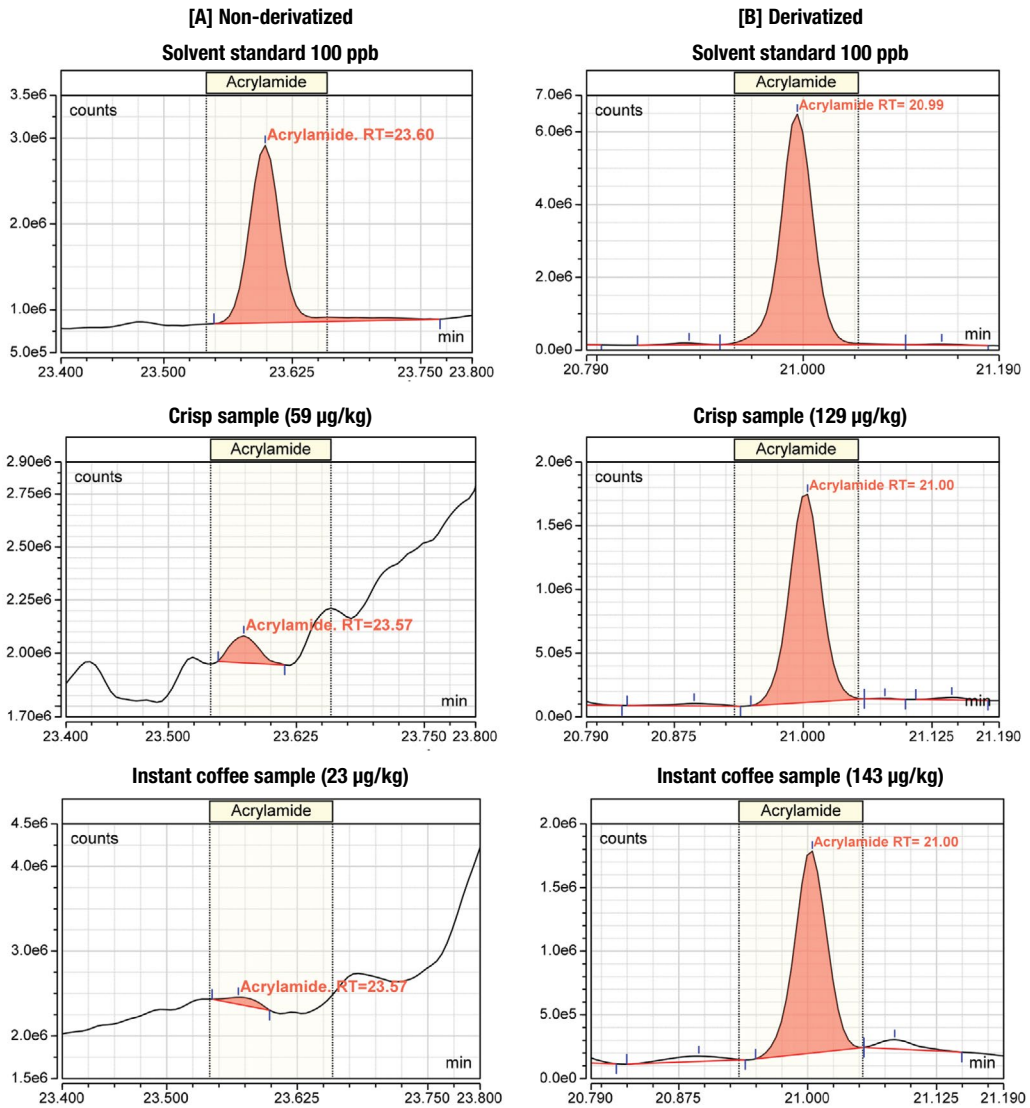

Figure 7. Examples of chromatographic selectivity of the same acrylamide calibration working standard (100 ppb), crisp and instant coffee samples, A: non-derivatized (m/z 55), and B: derivatized with MSTFA + 1% TMCS (*m/z* 128). Sample results quoted using standard addition calibration.

Quantification of acrylamide in food samples

Samples of potato 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-labeled internal standard. A summary of results for potato crisps, instant and ground coffee samples is shown in Figure 8.

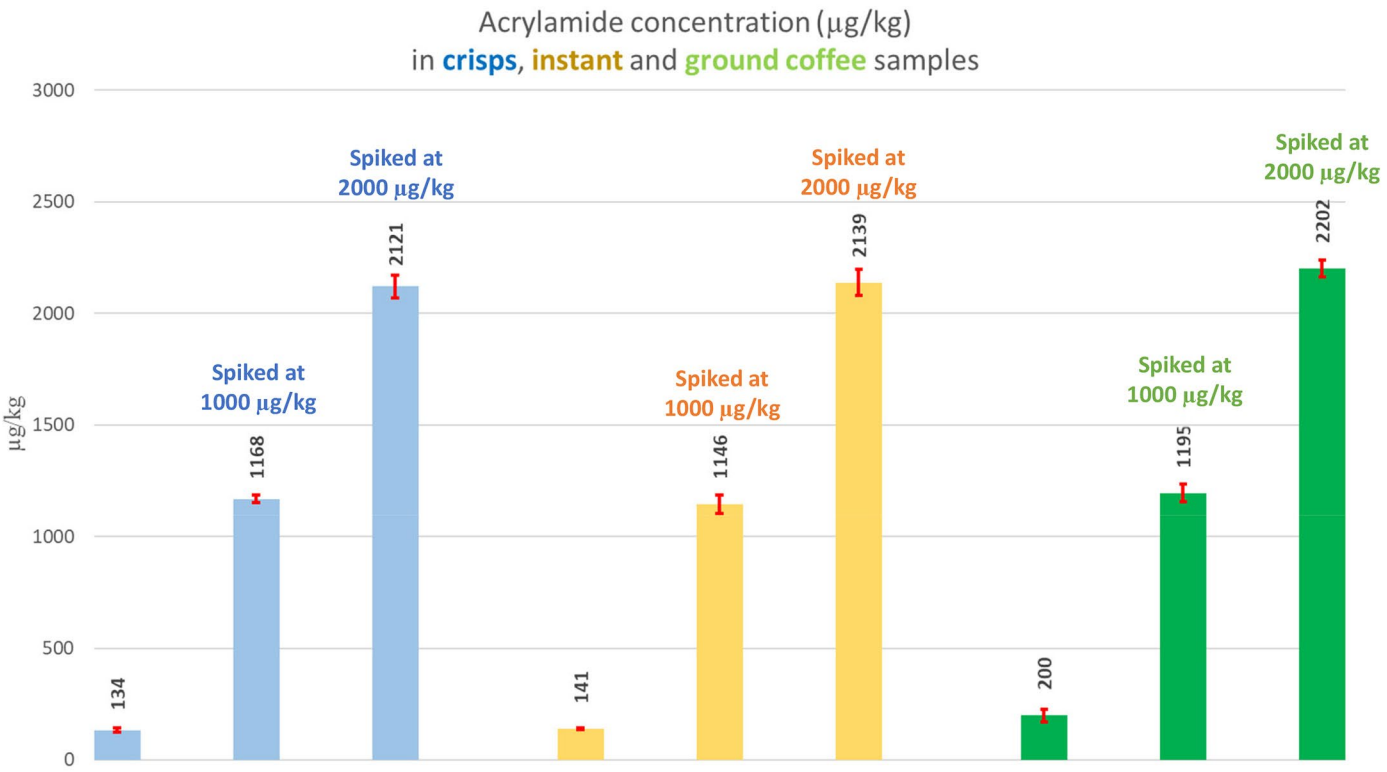

Figure 8. Average concentration of acrylamide (n=3) using standard addition calibration determined for unspiked and spiked (1000 and 2000 µg/kg) potato crisp, and instant and ground coffee samples, showing consistency at low and high levels. Standard deviation calculated from the three replicates is annotated, demonstrating the repeatability of the method.

Conclusion

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 and 1.01), and peak width (+10%) ≤4 s was achieved.
- Compound linearity obtained for derivatized acrylamide over a calibration range of 1 to 1000 ppb resulted in an 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 samples 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 potato 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.

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- Robustness of acrylamide responses in matrix was assessed by analyzing spiked ground coffee samples, mid and late during 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.
- Acylamide quantification using standard addition calibration eliminated the need for an expensive ^{13}C-labeled internal standard. The results illustrated consistency at low to high levels.
- Silylation of food and coffee samples extracted with acetonitrile, quantified in t-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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