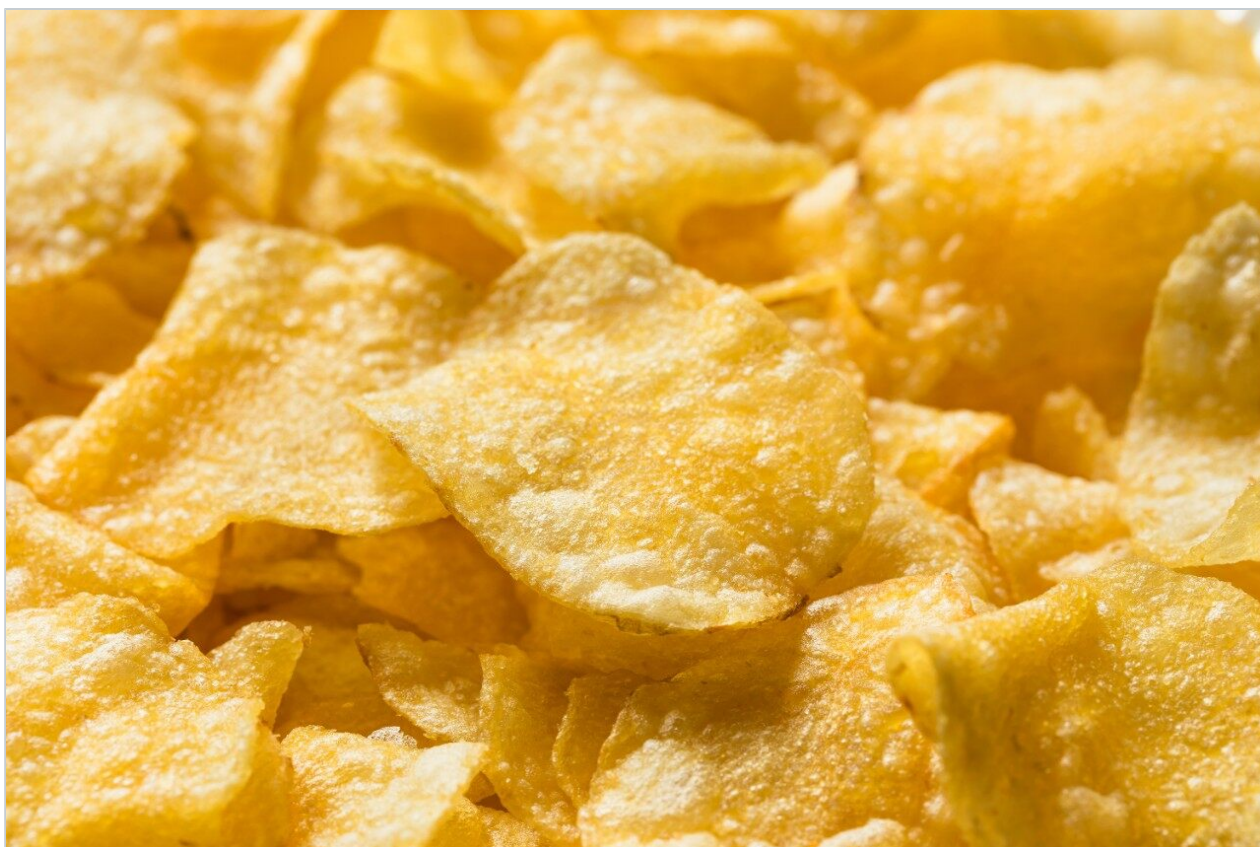


Application Note

Investigation of the Xevo TQ-S cronos System's Robustness for the Determination of Acrylamide in Processed Potato Chips

Renata Jandova, Sara Stead, David Gordon

Waters Corporation



Abstract

This application note shows the performance method for determination of acrylamide in processed foods by UPLC-MS/MS on an ACQUITY UPLC I-Class PLUS System coupled to the Xevo TQ-S cronos. Calibration characteristics, linearity, and sensitivity were all shown to be suitable for monitoring mitigation measures and checking compliance with EU benchmark levels for acrylamide in potato chips.

Benefits

Reliable, quantitative process for the routine analysis of contaminants in a processed food commodity that combines QuEChERS and SPE pass-through sample preparation for compliance with industry standards. Demonstrating robust performance in complex matrices and maximizing instrument uptime with minimal requirements for operator intervention over an extended analysis.

Introduction

The Xevo TQ-S cronos Triple Quadrupole Mass Spectrometer was developed as a reliable system for routine quantitative analysis, incorporating sample cone design elements that have been previously utilized in the ACQUITY QDa Mass Detector. As part of the reverse cone design, the narrowest restriction is in the center of the cone, whereas the entrance to the cone is comparatively wide (Figure 1). This design ensures that sample matrix and mobile phase buffer salts will not aggregate and block the orifice, therefore increasing the uptime of the instrument between cone cleans and providing reliable sensitivity with food matrices.

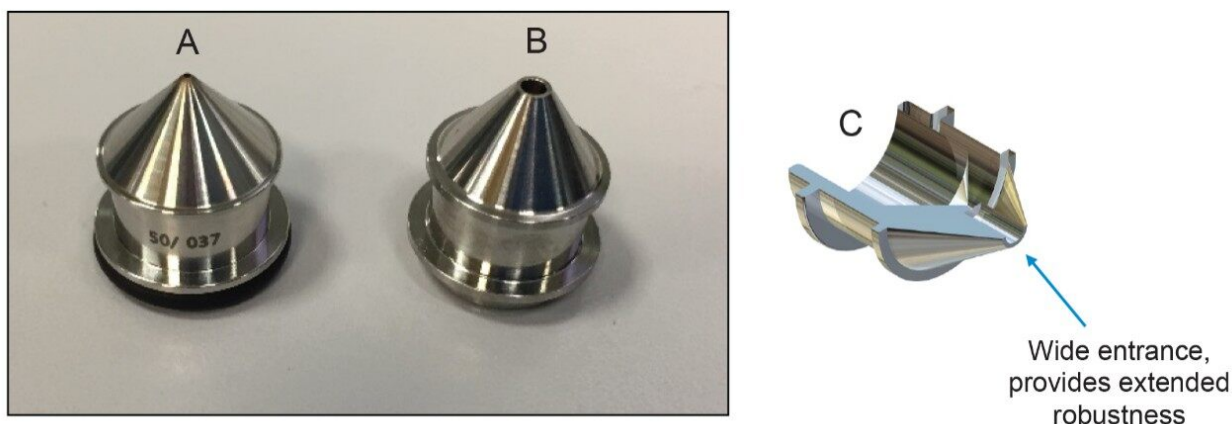


Figure 1. Conventional sample cone (A) and the reversed geometry sample cone design (B) and cross-sectional view of the reverse cone (C) showing the wider outer orifice (1.5 mm) and narrower inner cone regions (0.25 mm).

In addition to the reverse cone design, established technology also contributes to the robust performance of the Xevo TQ-S cronos, for example: 1) Orthogonal geometry ion source – this unique use of the dual orthogonal geometry enables efficient transmission of ions into the analyser at the same time as removing non-ionized materials (neutrals); 2) StepWave ion guide – the offaxis ion guide uses technology which removes neutral species and gas load, with the ion beam being actively extracted into a parallel “off axis” ion tunnel, which results in improved transmission to enable focusing into the analyzer, enhancing both sensitivity and robustness; 3) Collision cell technology – the use of travelling waves reduces the residence time of ions in the collision cell while further focusing the ion beam for improved performance, which enables rapid multi-component MRM data acquisition without loss in signal intensity while minimizing cross talk between adjacent MRM channels. Collision cell technology also ensures full compatibility with the high data acquisition rates required for high-quality, multi-component UPLC-MS/MS quantitative analysis.

A quantitative method for the determination of acrylamide was previously reported using the Xevo TQ-S micro System¹ and was shown to be applicable for a range of processed foods, including potato chips, bread, coffee, and baby foods. Acrylamide is a low molecular weight, highly water-soluble, organic compound that forms from the naturally occurring constituents (asparagine and sugars) in certain foods when prepared at temperatures typically higher than 120 °C. It forms mainly in baked or fried carbohydrate-rich foods where raw materials contain its precursors, such as cereals, potatoes, and coffee beans. Here, we demonstrate that the Xevo TQ-S cronos, with the inclusion of an enhanced sample cleanup, is a reliable system for routine quantitative measurements of challenging analytes, such as acrylamide, in complex food matrices operating

under typical conditions encountered in residue monitoring laboratories.

Experimental

Sample preparation

Samples of processed, coarsely-ground, dehydrated potato chips, previously characterized using GC-MS and found to contain levels of acrylamide below the EU benchmark level² (EU Regulation 2017/2158), were provided by a U.K.-based potato chip manufacturer.

Samples were extracted and cleaned up using the Waters Acrylamide UHPLC Enhanced Clean Up Kit (p/n: 176004423).

UPLC-MS/MS

UPLC system:	ACQUITY UPLC I-Class PLUS
Column:	ACQUITY UPLC HSS C ₁₈ SB, 100 Å, 1.8 µm, 2.1 × 100 mm) [p/n:186004119]
Mobile phase A:	Water with 0.1% formic acid (LC-MS-grade)
Mobile phase B:	Methanol (LC-MS-grade)
Flow rate:	0.2 mL/min
Injection volume:	5 µL (partial loop with needle overfill)
Column temp.:	30 °C
Sample temp.:	10 °C
Gradient:	Full gradient conditions available on request at https://www.waters.com/

MS instrument:	Xevo TQ-S cronos Triple Quadrupole
Ionization:	ESI+
Acquisition mode:	MRM
Capillary voltage:	+0.5 kV
Cone voltage:	20 V
Desolvation temp.:	600 °C
Desolvation gas flow:	1000 L/Hr
Source temp.:	150 °C

Compound	MRM transition (m/z)	Collision energy (eV)	Retention time (min)
Acrylamide	72.1 > 55.1	12	
Acrylamide	72.1 > 44.1	10	2.69
Acrylamide	72.1 > 27.1	10	
Acrylamide d3	75.0 > 58.1	15	2.66

Table 1. MRM transitions for acrylamide and acrylamide-d3 internal standard.

The MRM transitions that showed the best selectivity were used. Data were acquired using MassLynx Software v4.2 and processed using the TargetLynx XS Application Manager. The optimum dwell time was set automatically using the Auto-Dwell function.

The following source parameters were optimized on the Xevo TQ-S cronos during the method setup phase in favor of enhanced measurement repeatability.

1) ESI probe positioning

For the reverse cone design, the ESI probe was found to optimize at close proximity to the outer cone (wider cone entrance region) for maximum signal intensity. Adjusting the probe position further away from the optimal signal intensity position was found to reduce signal intensity but gave overall improvements in S:N and linear dynamic range due to reduced charge competition effects.

ESI probe position (relative to the outer cone)	Mean normalized % TIC intensity (n = 6) Acrylamide (<i>m/z</i> 72.1 > 55.1)	S:N RMS
5.4 mm (closest)	100	680:1
5.9 mm (mid)	70	681:1
6.4 mm (furthest)	60	930:1

Table 2. Effect of ESI probe position in relation to the reverse cone design on signal intensity and S:N in potato chip extract.

The ESI probe position closest to the outer cone region (5.4 mm) was selected for the measurement robustness study as the most challenging setting in terms of matrix load impacting the outer cone.

2) Cone gas flow

The nitrogen cone gas flow rate was also evaluated prior to commencing the robustness study. Due to the reverse cone design and smaller gas limiting orifice, lower cone gas flow rates are recommended for the Xevo TQ-S cronos, compared to the larger sample cone instruments. The outer cone shields the inner sampling cone, preventing buildup of matrix and/or mobile phase additives and allowing lower cone gas flow to be used while maintaining optimum performance and robustness.

Replicate injections of a potato chip extract containing acrylamide (n = 6) were performed under different cone gas flow rates to determine the optimum setting in terms of signal intensity and repeatability (%RSD).

Cone gas flow rate (L/Hr)	Mean normalized % TIC intensity Acrylamide (m/z 72 > 55)	%RSD TIC intensity (n = 6)
0	100	2.21
20	100	1.00
50	99.0	0.57
100	92.0	1.60
150	77.1	1.16

Table 3. Investigation of the effect of nitrogen cone gas flow rate, mean signal intensity, and repeatability.

A cone gas flow rate of 0 L/Hr was selected for the measurement robustness study as the most challenging setting in terms of matrix buildup on the cone.

Results and Discussion

Characterization of the Matrix

Prior to commencing the robustness study, the dehydrated potato chip samples were characterized using the method conditions described above to determine the concentration of acrylamide and provide an estimation of the matrix effects and potential interferences. A representative MRM chromatogram for acrylamide in potato chips is shown in Figure 2, indicating the quantifier and qualifier ion transitions are free from matrix interferences following the cleanup procedure.

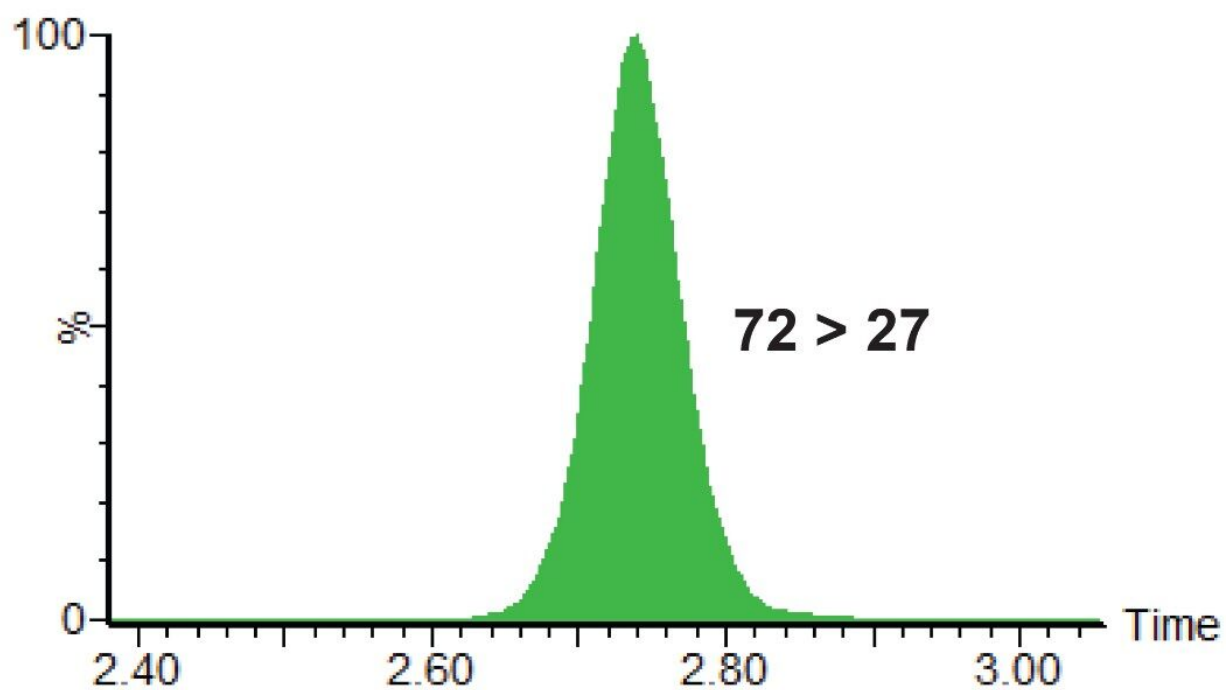
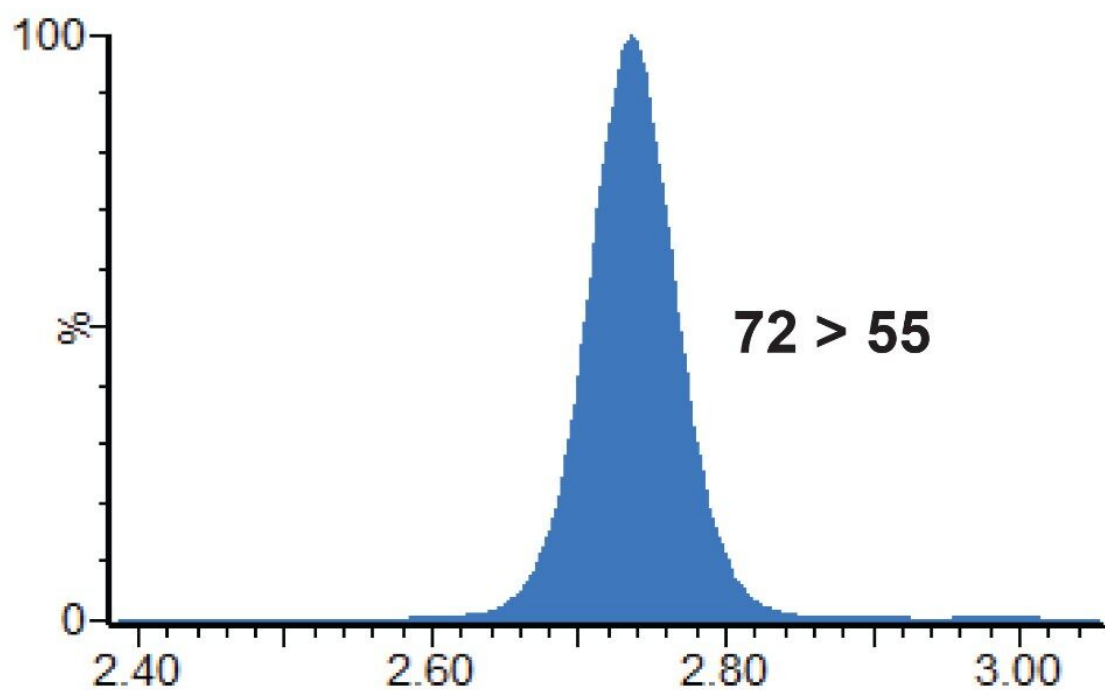


Figure 2. MRM chromatograms for acrylamide quantitative ion (72 > 55) and qualifier ion (72 > 27) in a potato chip sample.

For quantification purposes, a solvent calibration curve containing acrylamide-d3 was prepared over the

range of 2–500 ng mL⁻¹ inclusive of concentration levels equivalent to EU 2017/2158 performance requirements in potato chip matrix for LOD and LOQ of 15 and 50 µg kg⁻¹ respectively. As shown in Figure 3, signal suppression in the region of 40% was observed in potato chip extract as determined via comparison of the response of acrylamide-d3 when spiked into solvent and the matrix.

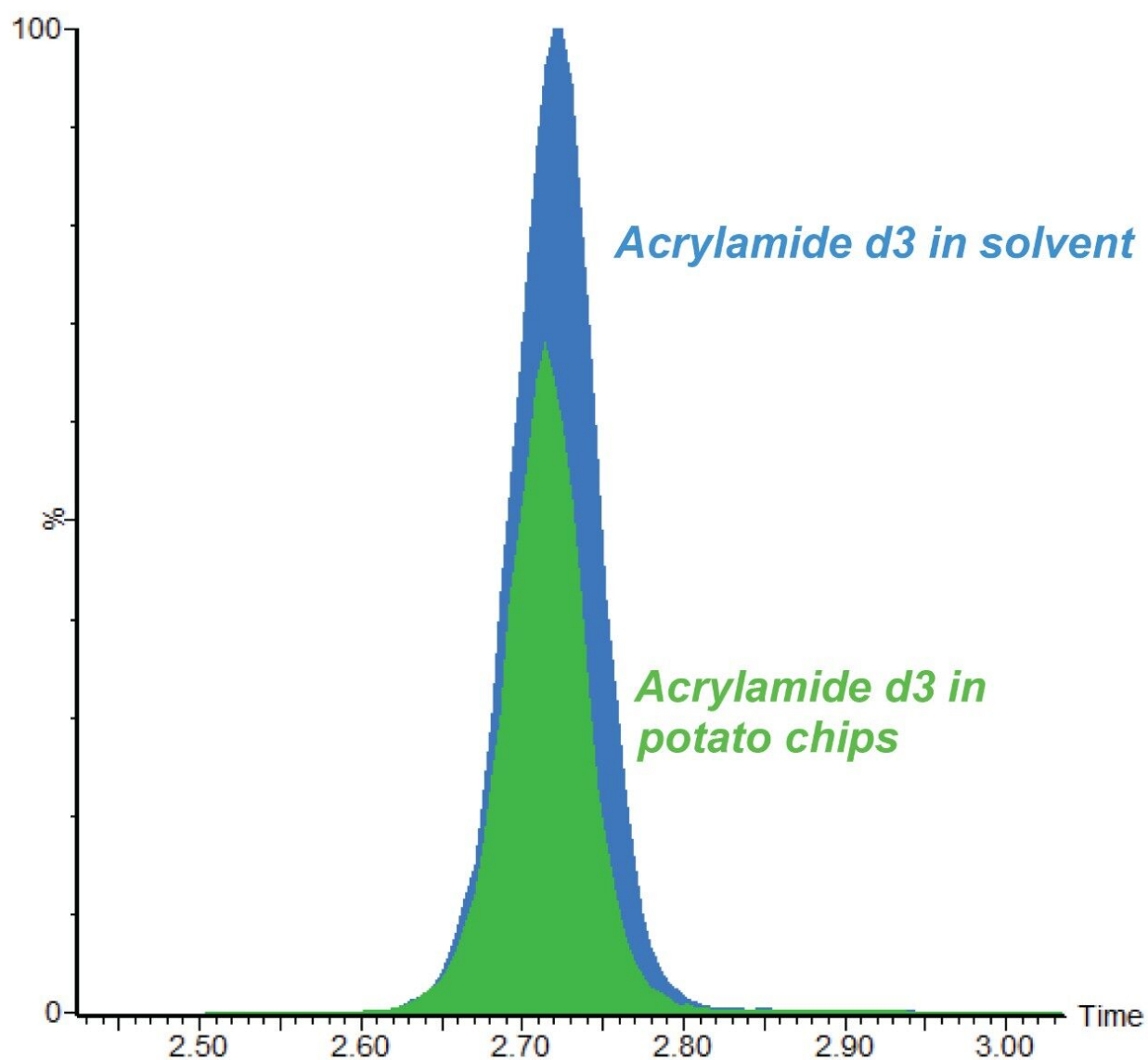


Figure 3. MRM chromatograms for acrylamide-d3 (75 > 58) in a potato chip sample and solvent standard spiked at 50 ng mL⁻¹.

Measurement Robustness

The robustness of the Xevo TQ-S cronos following repeated injection of an extract of potato chip (sample

268) previously found to contain acrylamide at $450 \mu\text{g kg}^{-1}$ was investigated. The source conditions used for the purpose of this study were found to improve the signal intensity and contribute towards the low %RSD obtained for repeated measurements. The results of the ESI probe positioning experiment show that improvements in S:N can be achieved when the probe is moved away from the cone in relation to the optimized position for maximum signal intensity. The cone gas flow rate experiment revealed that signal intensity remains stable at flow rates up to 50 L/hr, with the advantage of reducing the buildup of matrix/solvent contaminants on the cone surfaces.

The extract was split between four vials and systematic injections were made, maintaining the same mobile phase solutions and conditions for more than 500 injections ($5 \mu\text{L}$) with no user intervention. This represents a continuous running time equal to 83.3 hours (3.4 days) and total loading of over 1.5 mg of co-extractives following the 526 injections until the batch of mobile phase ran out. The peak area response for the both the analyte (acrylamide) quantifier transition ($m/z 72 > 55$) and the internal standard (acrylamide- d_3) ($m/z 75 > 58$) were plotted and were found to be within two standard deviations of the running mean response. A control chart showing the peak area for the quantitative ion transitions for acrylamide and the d_3 analogue was plotted in TrendPlot (Figure 4). An overall %RSD of ≤ 2.2 was obtained over the course of 526 injections.

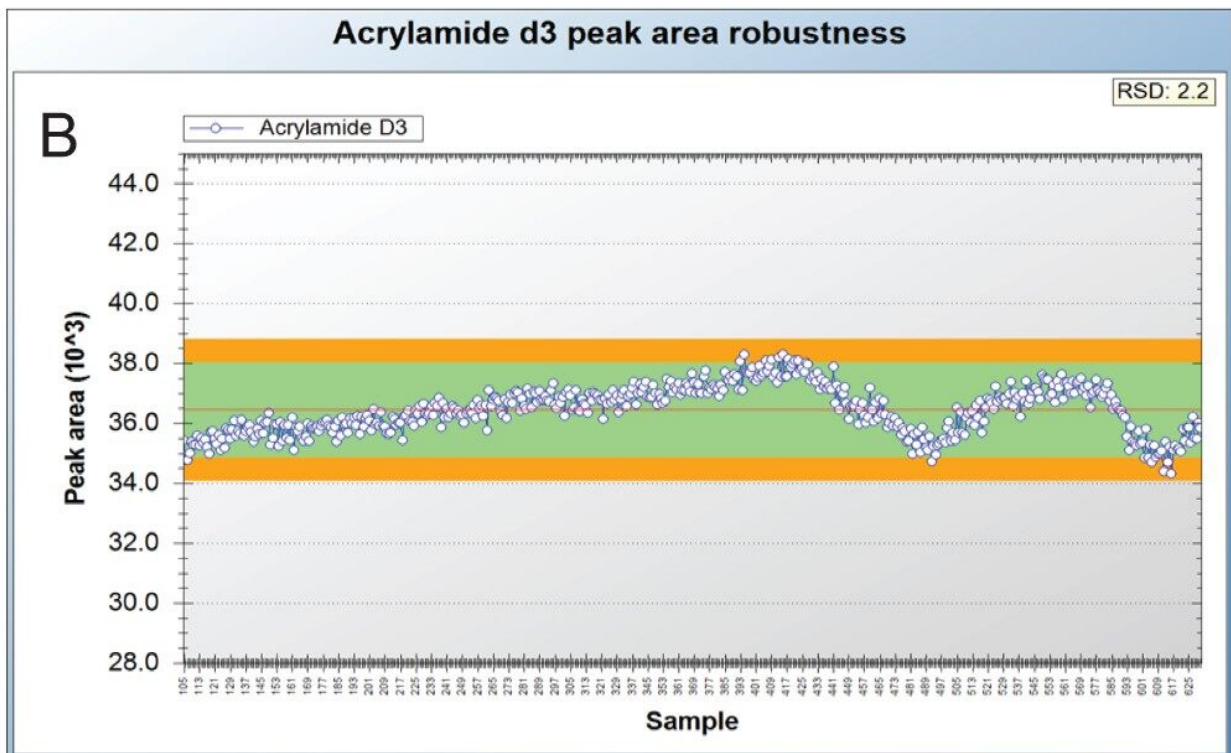
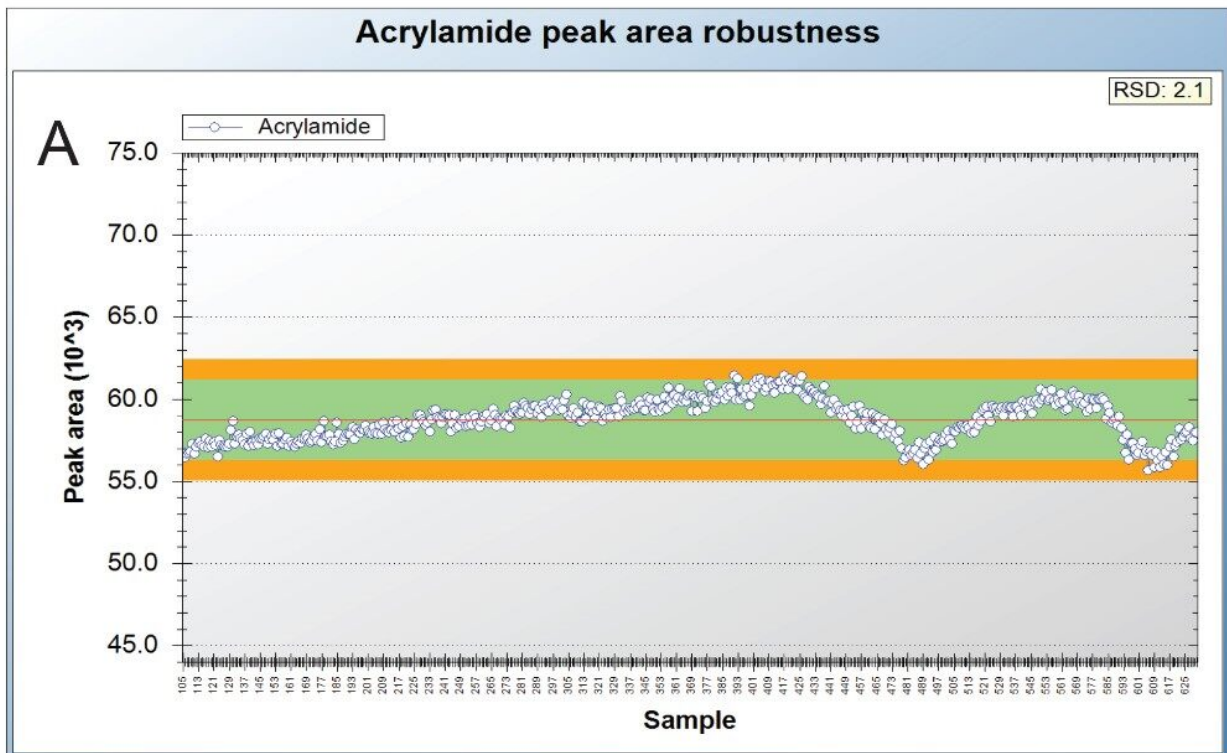


Figure 4. (A) Acrylamide ($ES+ 72 > 55$) and (B) acrylamide-d3 ($ES+ 75 > 58$) peak area repeatability following >500 consecutive injections in a processed potato chip matrix containing $450 \mu\text{g kg}^{-1}$; representing more

than 83 hours of continuous analysis time without operator intervention.

Conclusion

This application note shows the performance method for determination of acrylamide in processed foods by UPLC-MS/MS on an ACQUITY UPLC I-Class PLUS System coupled to the Xevo TQ-S cronos. Calibration characteristics, linearity, and sensitivity were all shown to be suitable for monitoring mitigation measures and checking compliance with EU benchmark levels for acrylamide in potato chips.

The robustness study was performed under the most extreme source parameters* to challenge the instrument under heavy matrix load. The results of the robustness study show the Xevo TQ-S cronos to be reliable for routine operation, without operator intervention during extended periods of analysis even for challenging small, polar analytes in complex food matrices. This superior performance can be attributed to the technology features of the Xevo TQ-S cronos, including the reverse cone design (shielding the inner cone from contamination), orthogonal geometry ion source, StepWave ion guide, and the T-Wave enabled collision cell.

*For routine operation the source parameters (ESI probe position and cone gas flow rate) are recommended to be optimized in favor of S:N with the benefit of reducing matrix buildup on the outer cone surface.

References

1. Determination of acrylamide in processed foods using the ACQUITY UPLC I-Class System and Xevo TQ-S micro Application Note Waters Corporation, 720006495EN (2019).
2. EU Regulation 2017/2158 Establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. (2017) L 304/24–44 *Official Journal of EU*.

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