

Application Note

## Determination of Acrylamide: Are You Avoiding Isobaric Interferences?

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Janitha De-Alwis, Euan Ross, Stuart Adams

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

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## Abstract

EU legislation (Commission Regulation (EU) 2017/2158) established benchmark levels of acrylamide in processed food with the onus on food producers to establish mitigation measures if they are exceeded. Waters developed an accurate and robust acrylamide method utilizing a fast, effective extraction and clean-up approach coupled with determination by LC-MS/MS. In response to recently reported issues with N-acetyl- $\beta$ -alanine, 3-aminopropanamide, and lactamide potentially causing overestimation of acrylamide levels in food, the selectivity of our approach was investigated further. It was confirmed that these three compounds would undergo in-source fragmentation to produce an ion with an identical  $m/z$  to acrylamide and this would subsequently fragment to daughter ions used for the determination of acrylamide. The investigation into sample clean-up showed that these compounds were not removed from the sample extracts and if not chromatographically resolved then would interfere with the determination of acrylamide. The 3 interfering compounds are all chromatographically resolved using the previously published LC method. This was verified using FAPAS reference materials where the results generated showed close agreement with the certified value. This is supported by the results in the original application note for the FAPAS reference materials that showed close agreement with the certified value.

## Benefits

- A rapid, accurate, precise, and cost-effective approach to quantifying acrylamide in processed food matrices including potato chips, coffee, bread, and baby food
- Chromatographic separation of potential interferences N-acetyl- $\beta$ -alanine, 3-aminopropanamide, and lactamide from acrylamide

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## Introduction

Acrylamide is a highly polar, water soluble compound and is formed during food production by high temperature (+120 °C) cooking with the main chemical reaction that causes this known as the Maillard Reaction.<sup>1</sup> The levels in food are of concern due to the toxicological properties which include neurotoxicity, genotoxicity, carcinogenicity, and reproductive toxicity.<sup>1</sup> As acrylamide is present in a wide range of everyday foodstuffs, an accurate and robust method that can detect and quantify low levels of acrylamide is vital. EU regulation 2017/2158 establishes mitigation measures and benchmark levels for reducing the presence of acrylamide in food.<sup>2</sup>

Our latest solution for determination of acrylamide in complex food matrices such as potato chips and coffee, utilizes a modified QuEChERS extraction procedure with two clean-up options, dispersive SPE or Oasis MCX pass through SPE for enhanced clean-up, prior to LC-MS/MS using an ACQUITY UPLC HSS C<sub>18</sub> SB Column.<sup>3</sup>

A recent study has identified the presence of other compounds that can interfere with the quantification of acrylamide in various food matrices such as coffee.<sup>4</sup> Potential isobaric impurities of acrylamide were studied and an in-source fragment of N-acetyl-β-alanine was identified as the main isobaric ion. 3-aminopropanamide and lactamide were some of the other notable interferences to be reported. Although our methodology had gone through vigorous testing as part of the validation, in light of these recent findings regarding possible isobaric interference, we re-investigated the selectivity of our method.

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## Results and Discussion

This study was undertaken to ensure that the Waters Acrylamide Kits were not overestimating acrylamide residue levels based on this new information. Three aspects were investigated; whether the compounds generated transitions that were isobaric with those used for acrylamide, whether the protocols used for sample clean-up removed these compounds, and whether the potential interferences were chromatographically separated using the conditions published in the Waters application note [720006495EN](#).

An investigation of potential in-source fragmentation of the three compounds using RADAR scans was performed on solvent standards. RADAR is an acquisition mode that acquires both MRM and full scan MS simultaneously, without any significant loss in performance, a unique capability that can both simplify and accelerate method development. RADAR is a standard feature of all Xevo Tandem Quadrupole Mass Spectrometer Systems. These scans (Figure 1) confirmed that the compounds did indeed generate ions at  $m/z$  72 in the source when using the MS parameters from the acrylamide method. Subsequent product ion scans for N-acetyl- $\beta$ -alanine, 3-aminopropanamide, and lactamide, using  $m/z$  72 as the precursor ion, confirmed the formation of product ions at  $m/z$  55 and 27. Hence these compounds have the potential to provide interference on the 2 transitions that are used for the detection of acrylamide ( $m/z$  72>55 and 72>27) and lead to overestimation of measured acrylamide concentrations.

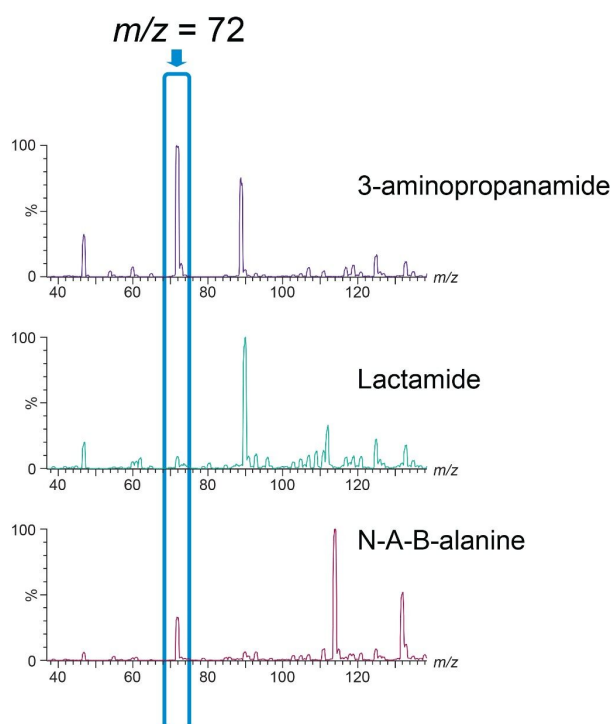


Figure 1. Mass spectra from the RADAR acquisition for lactamide, 3 aminopropanamide, and N-A-β-alanine.

We investigated whether the two clean-up options successfully removed the potential interferences and whether these compounds co-eluted with acrylamide using the published LC conditions. The results obtained from the initial investigation, as demonstrated in the chromatograms in Figure 2, indicated that these compounds were not removed completely from the extracts after clean-up so chromatographic separation was essential. Figure 3 shows that when using the previously published conditions with the ACQUITY HSS C<sub>18</sub> SB Column and pH controlled mobile phase, N-acetyl-β-alanine, 3-aminopropanamide, and lactamide were all well resolved from the acrylamide peak. When formic acid was removed from the mobile phase A (pH=7), the retention time of the acrylamide and lactamide peaks were not affected, remaining at 2.90 and 1.90 min respectively. However, N-acetyl-β-alanine peak shifted from 2.95 to 2 mins while 3-aminopropanamide was not retained (Figure 4). Therefore, it can be suggested that N-acetyl-β-alanine could co-elute with acrylamide if the pH of the mobile phase was not controlled.

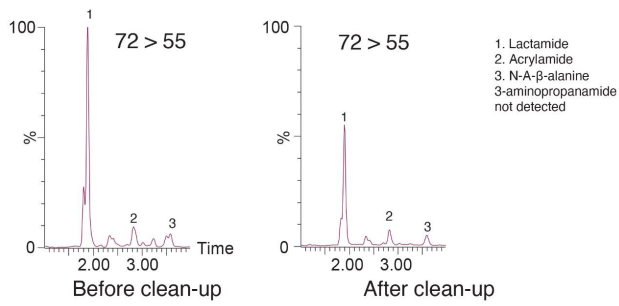


Figure 2. Chromatograms from analysis of coffee extracts before and after clean-up with Oasis MCX.

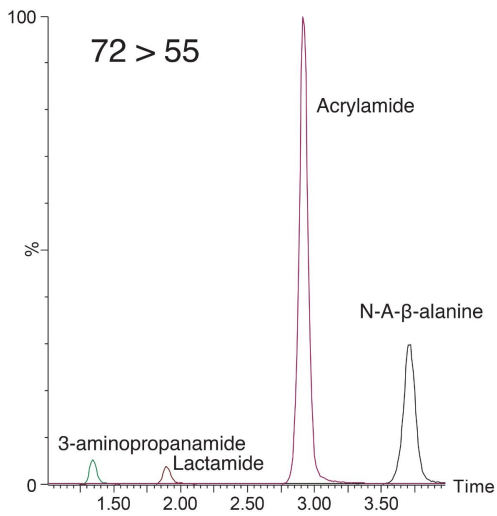
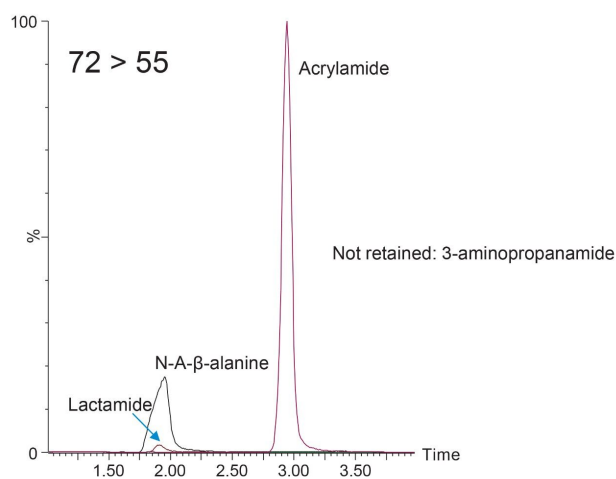


Figure 3. Chromatographic separation of 3-aminopropanamide, lactamide, acrylamide, and N-A-β-alanine by current Waters methodology.

Mobile phase A: 0.1% Formic acid in water,  
B: Methanol



*Figure 4. Chromatographic separation of 3-aminopropanamide, lactamide, acrylamide, and N-A-β-alanine with modified mobile phase.*

*Mobile phase A: Water, B: Methanol*

To confirm that using Waters' Acrylamide Kits were not overestimating acrylamide residue levels, we analysed a coffee reference material purchased from FAPAS (Ref: TYG010RM). The results obtained, with dSPE ( $\bar{x} = 248 \mu\text{g}/\text{kg}$ ) and Oasis MCX SPE clean-up ( $\bar{x} = 250 \mu\text{g}/\text{kg}$ ) are in good agreement with the assigned value,  $249 \mu\text{g}/\text{kg}$ . The results show the correct assignment of the acrylamide peak in chromatograms from analysis of the reference material in the presence of the other compounds. The selectivity of the method using ACQUITY HSS C<sub>18</sub> SB Column with optimized pH, enabled the accurate quantification of acrylamide. The ion ratios and retention times from the analysis of the reference materials agreed well with the reference values derived from the spiked samples and all were well within the tolerances typically used for residue analysis (e.g.  $\leq 20\%$  and  $\pm 0.1$  minutes).

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## Conclusion

The Acrylamide Kit method is providing accurate measurements for acrylamide in FAPAS reference materials even with the presence of N-acetyl- $\beta$ -alanine, 3-aminopropanamide, and lactamide. The critical parameter is to control the pH of the aqueous mobile phase to ensure separation of the 3 potential interferences from acrylamide.



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## References

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