GC/MS of Acrylamide in Air-popped Potato Snacks with Bond Elut C18 Sample Prep

Application Note

Food Testing & Agriculture

Author

Pat Sasso
Agilent Technologies, Inc.

Abstract

In the decade since the discovery and mechanism of its formation, acrylamide levels have been monitored and reported for data gathering purposes by regulatory agencies. This note describes a simple cleanup procedure using solid phase extraction (SPE), coupled to GC/MS on an Agilent J&W VF-WAXms GC column, to measure low levels of acrylamide in newly introduced potato snacks processed using heat and pressure (air-popping). Agilent Bond Elut SPE cartridges and VF-WAXms columns offer the lowest potential for interferences, making the assay more reliable for complex sample matrixes.

Agilent Technologies

Introduction

Even though Swedish researchers were first able to measure acrylamide in fried potato at alarmingly high levels, regulatory agencies have not introduced minimum acceptable requirements. This is mainly due to the fact that acrylamide at low dietary intake currently poses less of a health risk than the saturated fats and cholesterol also present in these food items. Many innovative commercial alternatives to deep-frying have been introduced recently. One such technology, air-popping, is being touted as a more health-conscious way to prepare starches such as potatoes. The combination of asparagine and starch in potatoes makes the formation of acrylamide more likely during heating. Recent enzymatic removal of asparagine has been tested and produced good results [1]. While LC/MS/MS using HILIC columns is widely accepted [2], it is equally acceptable to measure acrylamide in food items using GC/MS [3]. By leveraging sample preparation using acetone recovery, the technique presented here offers the laboratory flexibility to screen samples as an alternative to dispersive solid phase extraction (dSPE), also known as QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Simple) [4].

LC/MS/MS of acrylamide has a problem in that ions of interest are low mass and appear in the solvent cluster region, which is typically not optimized for detection but for the removal by electrospray sources. The use of a C18 SPE cartridge produces fewer low-mass interferences on the GC/MS TIC trace compared to others such as Florisil and polystyrene-divinylbenzene (PS-DVB). While the ions formed by acrylamide in electron impact are low mass and potentially subject to interferences, it is possible to use two of them (55, 71 amu) without encountering any problems related to the sample, or the sample prep materials. This sample preparation method is based on the draft U.S. Food and Drug Administration method [5]. The VF-WAXms phase, even after trimming off 5 m, provides better retention for polar analytes such as acrylamide. Other, less polar phases would offer less retention and a potential for interferences to co-elute.

Materials and Methods

An Agilent 7890A GC was coupled to an Agilent 5975C mass spectrometer with the inert EI 350 noncoated source. A VF-WAXms column was cut into two parts, with a 5-m section installed in a similar GC/FID used to evaluate the time required for adequate column bakeout at the end of the run. Acrylamide was obtained from Sigma-Aldrich Corp. (catalog no. A3553-100G).

Conditions

GC

Column:	Agilent J&W VF-WAXms, 30 m x 0.25 mm, 0.25 µm (trimmed to 25 m) (p/n CP9205)
Sample preparation:	Agilent Bond Elut C18, 1 g, 6 mL (p/n 14256001)
Sample:	1 g coarsely ground air-popped potato pieces
Carrier:	MSD helium, FID hydrogen for bakeout evaluation, both at 1 mL/min constant flow
Oven:	100 °C (hold 1 min), then to 200 °C at 5 °C/min, then to 260 °C at 25 °C/min (hold 15 min)
Injection:	Splitless, split vent on at 1 min (30 mL/min), gas saver on at 3 min (20 mL/min)
Inlet temperature:	150 °C
Detector:	FID for bakeout evaluation at 260 °C
MSD transfer aux Bond Elut:	260 °C
GC:	Agilent 7890A GC
Sampler:	Agilent 7693A Automatic Liquid Sampler, 5 µL volume injection

MS

MS:	Agilent 5975C Series GC/MS with inert EI 350 source, tandem axis detector
Solvent delay:	7 min
MS temperature:	300 °C (source), 150 °C (quad)
SIM mode:	Mass 55.00, 71.00, dwell 100 ms for each

Agilent supplies (unless otherwise stated)

Drying tubes:	Pyrex centrifuge tubes, conical with screw cap, 15 mL, with graduations (Sigma-Aldrich Corp., catalog no. CLS808215-12EA)
Vials:	Amber screw cap (p/n 5182-0716)
Caps:	Blue screw cap (p/n 5282-0723)
Vial inserts:	Glass with polymer feet, 250 µL (p/n 5181-1270)
Syringe:	10 µL (p/n 5190-1483)
Septum:	Advanced Green non-stick (p/n5183-4759)
Inlet liner:	Direct connect liner (p/n G1544-80731)

Standards

Acrylamide standard solutions were prepared in acetone from 10 to 1,000 ng/mL, to demonstrate linearity and detection limit. Air-popped potato snacks were purchased from a local grocery store. After initial screening, it would also be possible to prepare a matrix-matched calibration set using the extracts as dilution solvent.

Sample preparation

1. Slurry 1 g coarsely ground air-popped potato in 4 mL HPLC acetone
2. Shake on wrist shaker for 30 min
3. Spiked samples at 100 ng/g (100 ppb) to demonstrate adequate recovery from the potato matrix
4. Centrifuge at 4,000 rpm for 5 min
5. Pre-condition Bond Elut C18 SPE tubes with 4 mL acetone, apply vacuum for 1 minute to remove excess acetone
6. Replace waste tube with collection tube
7. While passing supernatant through glass wool to filter, apply 2 mL to the pre-conditioned Bond Elut C18 cartridge and elute under gravity
8. Elute with additional 2 mL clean acetone under gravity
9. Evaporate eluate to 1 mL final volume
10. Transfer to vial for injection

Results and Discussion

As can be seen in Figure 1, the equipment used to recover acrylamide from samples does not introduce any co-eluting interference. This equipment includes the SPE apparatus, tubes and packing and associated plastic containers.

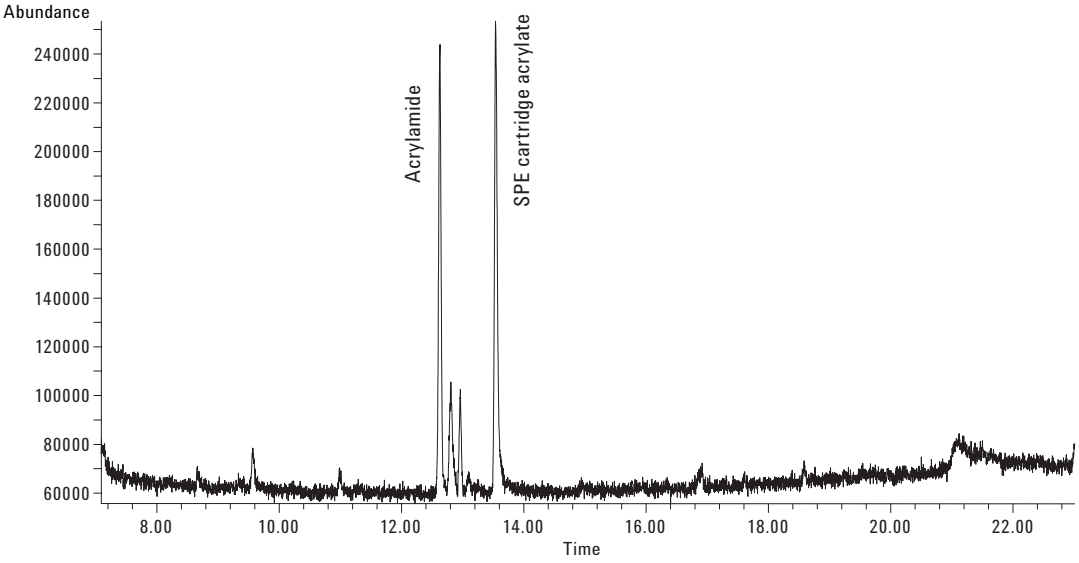

Figure 1. Scan mode 10 ng/g acrylamide recovery from SPE tube and filter showing trace acrylate with an ion at 55 amu that is closely eluting but not interfering. Silica-based SPE sorbents tended to have more low-mass interferences than the C18 shown here.

Figure 2 shows the co-extracted compounds in the air-popped matrix. This could include trace amounts of added preservatives or fatty acids from safflower oil used during addition of various flavorings. Samples are considerably cleaner after SPE versus liquid extraction in acetone.

Recovery of the acrylamide at the ng/g level from the spiked sample extraction can be seen in the SIM mode chromatogram in Figure 3. It is appropriate to mention here that a matrix-matched calibration could be prepared if initial screening showed no detectable levels.

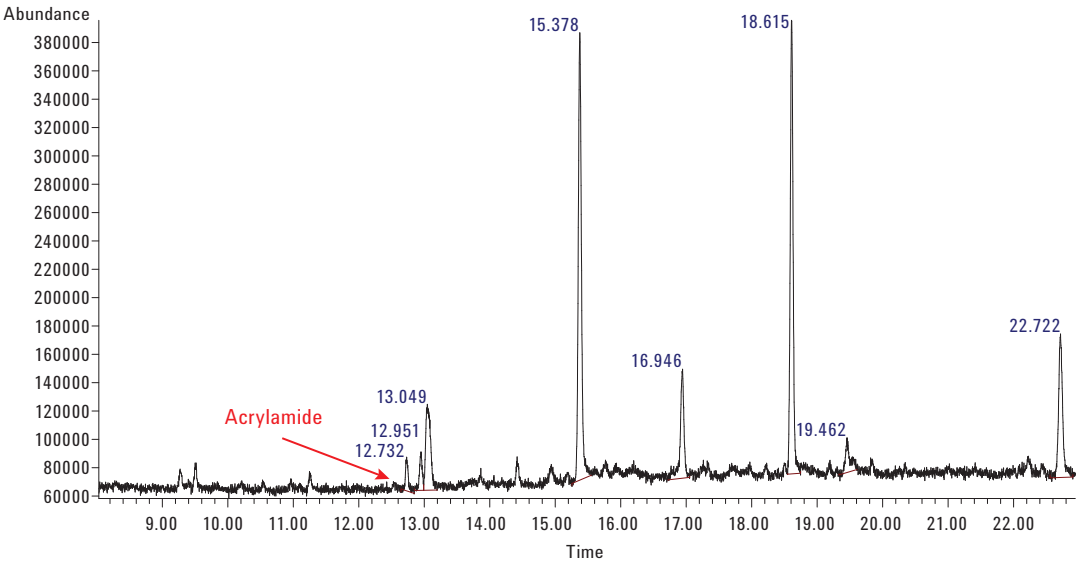

Figure 2. Scan mode of air-popped potato extract showing potential interferences on an Agilent J&W VF-WAXms GC column near elution time (12.6 min) of acrylamide, and a few high boiling solutes requiring bakeout.

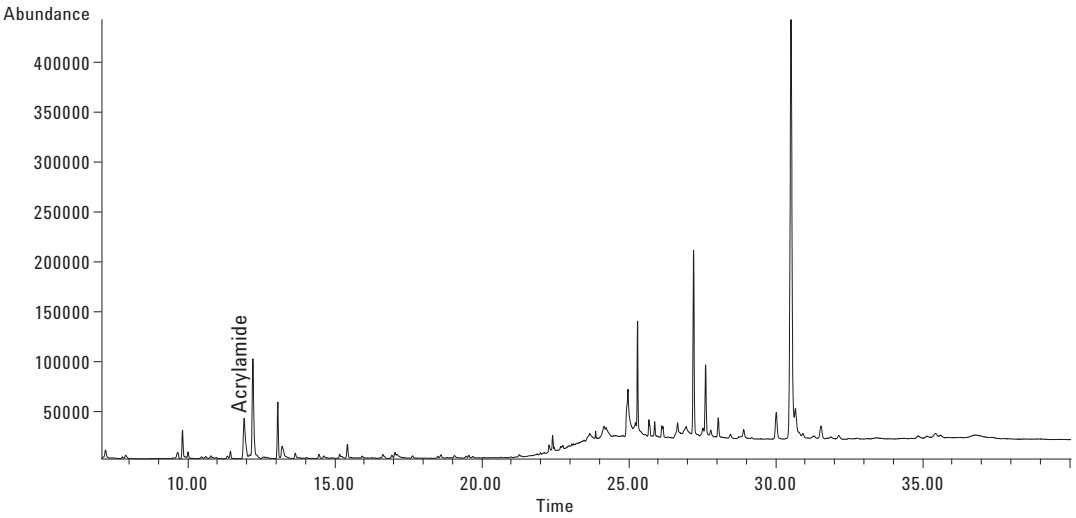

Figure 3. Spiked sample demonstrating separation of co-extracted fragment ions at 55 and 71 amu in SIM mode requiring column bakeout, on an Agilent J&W VF-WAXms GC column.

Recovery of spiked samples is excellent, making the use of Bond Elut C18 SPE a viable tool for low level isolation. One of the main challenges in sample preparation is drying down the extract, which can result in a slight change in retention time. The presence of matrix components in large excess relative to the acrylamide can cause some slight change in retention time. A 5-µL injection was needed to achieve the desired limit of quantitation.

Figure 4 is an overlay of the standard in acetone, which was replaced as the sample extraction solvent used in the original draft method produced by the U.S. FDA, since the samples are much lower in fat content than fried potatoes. A series of 10 sample injections shows that slight drift is present but reproducible from injection-to-injection, giving confidence that the VF-WAXms column is robust enough to analyze challenging complex matrixes such as these. Matrix-matched calibration sets would remove this drift, providing further confidence in the assay. It is also possible to incorporate a capillary flow technology (CFT) device to backflush the system, shortening the bakeout time.

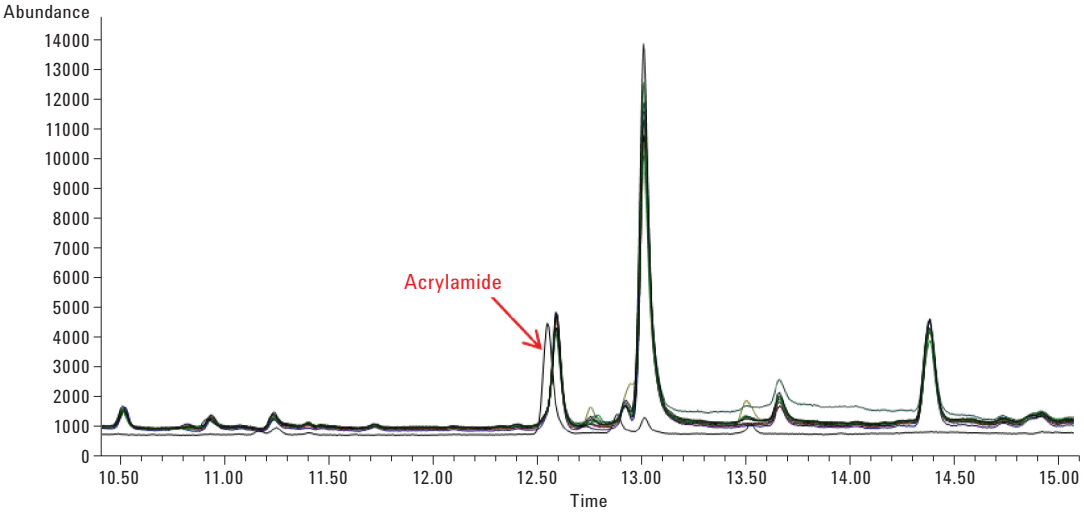

Figure 4. Typical sample set of 10 injections overlaid with demonstrated retention time shift due to matrix effect, highlighting robustness of the Agilent J&W VF-WAXms GC column, and good recovery from Agilent Bond Elut C18 SPE.

Conclusions

The Agilent J&W VF-WAXms column provides the separation power to resolve all the ions present that could potentially interfere with quantitation of acrylamide in an air-popped potato matrix. Leveraging an SPE cleanup on Agilent Bond Elut C18 enables screening in food laboratories concerned with finding alternative ways to prepare future innovations in heat-processed starches. After completion of a typical sample set, a simple routine maintenance will include replacing the inlet liner, which was dirty on inspection. It is also prudent to trim a few centimeters off the column head, since there are likely to be high-boiling solutes there as well. The mass spectrometer source remained clean and did not require maintenance.

References

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