



Determination of Acrylamide in Cooking Oil by Agilent Bond Elut QuEChERS Acrylamide Kit and HPLC-DAD

Application Note

Food Safety

Authors

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Abstract

This application note describes a method based on a quick, easy, cheap, effective, rugged and safe (QuEChERS) multiresidue sample preparation procedure. The QuEChERS method presents an extraction and cleanup protocol for the determination of acrylamide employing methacrylamide as the internal standard. The analyte and internal standard were separated on an Agilent ZORBAX HILIC Plus column (4.6 mm × 50 mm, 3.5 μm) by isocratic elution employing 3% 5 mM CH₃COOH and 97% CH₃CN with subsequent diode array detection at 210 nm. The acrylamide recoveries ranged from 84 to 93.8% with relative standard deviation of less than 4%. The limits of detection and quantification were 32.4 and 108 ng/mL respectively.



Agilent Technologies

Introduction

Acrylamide (Figure 1) is an organic compound used to manufacture plastic materials, paper, dyes, cosmetics and polyacrylamide, which is a water stabilizer for sewage water treatment and gel electrophoresis [1]. Acrylamide also occurs naturally as a byproduct of the cooking process and its presence in food was first confirmed by Swedish researchers in 2002 [2]. The Swedish findings about high levels of acrylamide in heat treated foods were quickly confirmed by the UK Food Standards Agency through its official website notification on 17 May 2002 and US Environmental Protection Agency (USEPA) found the limit for acrylamide in drinking water to be extremely low (0.5 µg/kg) [3]. Carbohydrate-rich foods such as french fries processed at high temperatures and under low moist conditions are of concern because high concentrations of acrylamides are produced in this process [4]. Acrylamide, at high concentrations, has adverse effects as a human neurotoxin and has also been classified as a probable carcinogen and genotoxicant [5]. Acrylamide vapors irritate the eyes and the skin and can cause paralysis of the cerebrospinal system [6].

The QuEChERS method, which was initially designed for the analysis of pesticides in food has since been adapted to include the extraction of a variety of analytes such as acrylamide [5]. In general, there are two major steps: extraction and dispersive SPE cleanup. The method uses a single step buffered acetonitrile extraction while simultaneously salting out water from the sample using anhydrous magnesium sulfate (MgSO_4) to induce liquid-liquid partitioning. After removing an aliquot from an organic layer for further cleanup, dispersive solid phase extraction (dSPE) is conducted using a combination of primary secondary amine (PSA) to remove fatty acids, and anhydrous MgSO_4 to reduce the remaining water in the extract.

This application note presents a method for the analysis of acrylamide in cooking oil with HPLC-DAD. The method includes sample preparation with Bond Elut QuEChERS Extraction kit for acrylamides (p/n 5982-5850) and Bond Elut EN Fruits and Vegetables with Fats and Waxes Dispersive SPE kit (p/n 5982-5156).

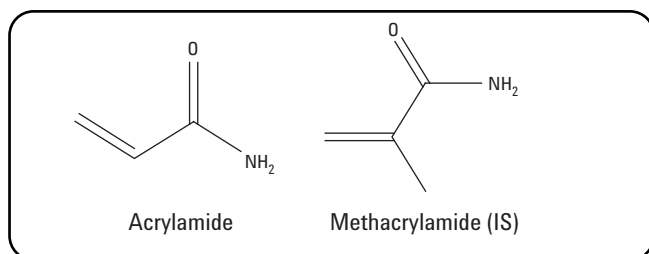


Figure 1. Chemical structures for acrylamide and methacrylamide (IS).

Experimental

Reagents and Chemicals

All reagents were analytical or HPLC grade. Acetonitrile (CH_3CN), n-hexane, acrylamide and methacrylamide were purchased from Sigma-Aldrich (St. Louis, MO, USA). The water used was from a MilliQ system from Millipore (Milford, Mass, USA). The mobile phase was filtered through a Whatman membrane filter (47 mm diameter and 2 µm pore size).

Standard Solutions

Standard stock solutions (1 mg/mL) were prepared by dissolving 10 mg of the acrylamide/methacrylamide in 10 mL MilliQ water and stored at 4 °C. All working solutions were prepared daily by serial dilution also in MilliQ water.

Equipment and Material

The analysis was performed on an Agilent 1200 Series HPLC, Agilent Technologies Inc. (Santa Rosa, CA, USA) equipped with a binary pump and a diode array detector (DAD) set at 210 nm. Separation of the compounds was achieved on an Agilent ZORBAX HILIC Plus column (4.6 mm × 50 mm, 3.5 µm, p/n 959943-901). The data was processed by Agilent ChemStation for LC/MS 2D system software.

Extraction and cleanup were achieved with Agilent Bond Elut QuEChERS Extraction kit for acrylamides, p/n 5982-5850 and Bond Elut QuEChERS EN Dispersive SPE kit, p/n 5982-5156.

Instrument conditions

HPLC conditions

Table 1. HPLC Conditions used for Separation of Acrylamide and Methacrylamide

Column	Agilent ZORBAX HILIC Plus 4.6 × 50 mm, 3.5 µm
Flow rate	0.2 mL/min
Column temperature	30 °C
Injection volume	5 µL
Mobile phase	Isocratic elution: A = 3% 5 mM acetic acid B = 97% acetonitrile
Run time	10 min
Post time	3 min
Detection	DAD @ 210 nm

Sample preparation

The cooking oil (sunflower-based) was purchased from a local store.

Extraction

Figure 2 outlines the extraction procedure. A 1-g sample of cooking oil was placed into a 50-mL centrifuge tube from the Bond Elut QuEChERS Extraction kit. Samples were spiked appropriately to yield working solutions for recoveries and reproducibility studies. Samples, with exception of the blank, were fortified with 1000 μ L spiking solution and mixed with 9 mL of water. After shaking vigorously for 1 min, 10 mL of CH_3CN were added, followed by an addition of Agilent Bond Elut QuEChERS extraction salt mixture for acrylamides (p/n 5082-5850). The QuEChERS extraction packet contained 4 g of anhydrous MgSO_4 and 0.5 g NaCl. Due to the high concentration of long chain fatty acids in the cooking oil, 5 mL of hexane were added to the extraction mixture. The sample tubes were hand-shaken vigorously for 1 min and then centrifuged at 4000 rpm for 5 min. Note that in the centrifuge tube, the hexane forms a third layer [hexane (top layer): acetonitrile (middle layer): water + salts (bottom layer)]. The hexane layer was discarded prior to the dSPE cleanup.

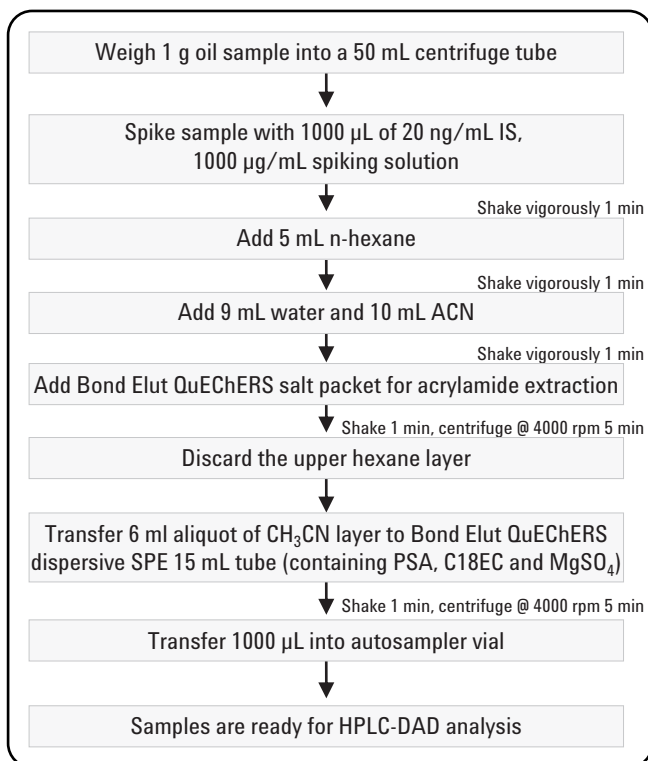


Figure 2. Flow chart for the QuEChERS sample preparation procedure.

Dispersive SPE cleanup

A 6-mL aliquot of the CH_3CN layer (now the top layer after hexane removal) was transferred into a Bond Elut QuEChERS EN Dispersive SPE 15 mL tube. The SPE tube contained 150 mg PSA, 150 mg C18EC and 900 mg MgSO_4 . The tubes were then further centrifuged at 4000 rpm for 5 min. A 1000- μ L amount of extract was placed in an autosampler vial for an HPLC-DAD analysis.

Results and Discussion

Chromatographic analysis

The separation of acrylamide and methacrylamide (internal standard) was achieved on an Agilent ZORBAX HILIC Plus column (4.6 mm \times 50 mm, 3.5 μ m, p/n 959943-901) using isocratic elution, with 3% 5 mM acetic acid and 97% acetonitrile as the mobile phase. The column temperature was set at 30 $^\circ\text{C}$ while the flow rate was set at 0.2 mL/min. Figure 3 shows a typical chromatogram for the injection of the standard mixture. Different mobile phase polarity compositions, from 100% water to 100% acetonitrile, were evaluated. The best retention with a short run time was obtained with 97% acetonitrile and 3% acetic acid.

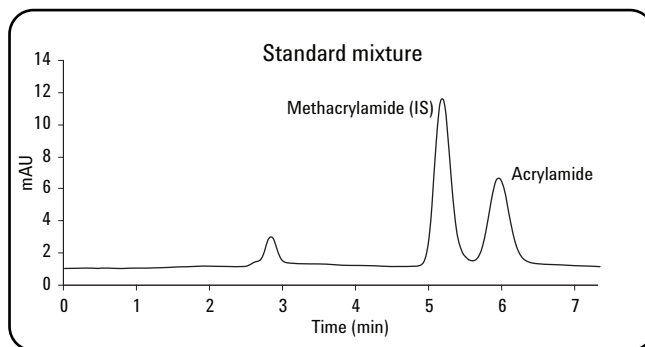


Figure 3. Chromatogram of the standard mixture of acrylamide and methacrylamide (IS).

QuEChERS method

An Agilent Bond Elut QuEChERS salt packet (p/n 5982–5850) containing 4 g MgSO_4 and 0.5 g NaCl was used to extract acrylamide from 1 g of cooking oil sample. The addition of the salt induced the acetonitrile–water phase separation [5]. A 5-mL volume of n-hexane was added to the samples for defatting, which removed long chain fatty acids that could create challenges in chromatographic analysis by giving peaks overlapping with the analyte or clogging the column [6]. Dispersive SPE was employed for sample cleanup.

The QuEChERS protocol in this application note is simple and does not require evaporating the extracting solvent. This is beneficial because acrylamides are usually lost during this step, leading to low recoveries. [7].

Linearity, limit of detection (LOD) and limit of quantification (LOQ)

Linearity

A linear calibration curve (Figure 4) was obtained by plotting the relative responses of analyte (peak area of analyte / peak area of IS) to the relative concentration of analyte (concentration of analyte / concentration of IS). The curve was generated by spiking the sample blanks at a concentration range of 0 - 1500 ng/mL. Good linearity was demonstrated with $r^2 = 0.9992$.

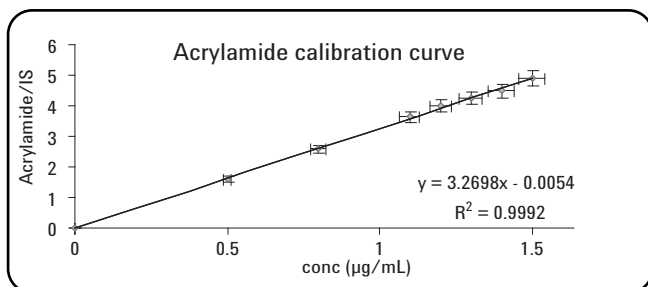


Figure 4. Acrylamide calibration curve.

Limits of Detection and Quantification

The limits of detection and quantification were evaluated from the concentration of acrylamide required to give a signal-to-noise ratio of 3 and 10 respectively. The limit of detection (LOD) was found to be 32.4 ng/mL while the limit of quantification (LOQ) was 108 ng/mL.

Recovery and Reproducibility

The recovery and reproducibility (RSD) were evaluated on spiked samples at three different fortification levels: 500, 1000, and 2000 ng/mL. The analysis was performed in replicates of six ($n = 6$) at each level. Table 2 shows the recoveries and RSD values for acrylamide.

Table 2. Recoveries and RSDs for the Acrylamide in Oil Sample ($n = 6$)

Concentration (ng/mL)	Recovery % ($n = 6$)	RSD % ($n = 6$)
500	84.0	3.2
1000	93.8	2.2
2000	92.2	1.5

The chromatograms of the oil blank and the spiked oil sample, after the QuEChERS extraction and cleanup, are shown in Figures 5 and 6 respectively. The blank oil extract did not show any detectable amounts of acrylamide. Similar results were reported on a study of acrylamide content in commercial frying oils [7]. The frying oils in that study did not contain any detectable amounts of acrylamide (detection limit of 0.02 µg/mL) prior to processing food samples.

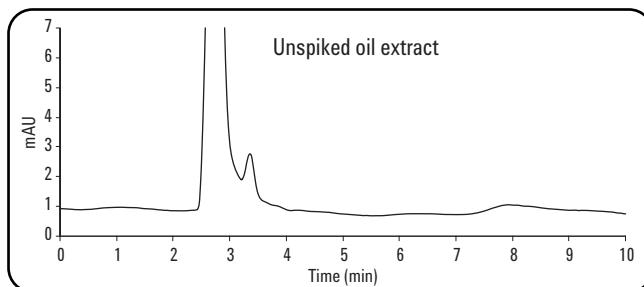


Figure 5. Chromatogram of the blank oil extract.

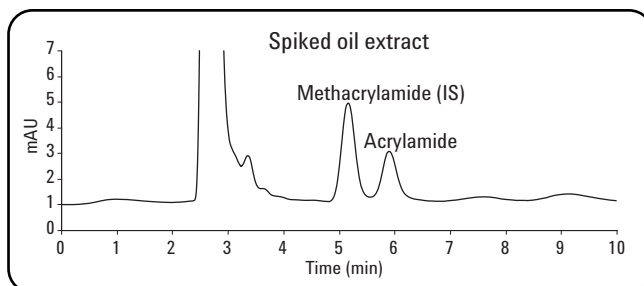


Figure 6. Chromatogram of the spiked oil extract.

Conclusions

A simple and fast multiresidue method using Agilent Bond Elut QuEChERS for acrylamide extraction and cleanup with an HPLC-DAD analysis on a HILIC column has been developed. High extraction yields with excellent RSD, LOD (32.4 ng/mL), and LOQ (108 ng/mL) were obtained. Therefore, the method may be applied for quality control of acrylamide in real samples.

References

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