Gas Chromatography/Mass Spectrometry Approaches to the Analysis of Acrylamide in Foods

Application

Food Safety

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Abstract

Discovery of acrylamide in cooked foods has required an examination of foods for potential exposure. A classic approach employs extracting acrylamide from the food with water and converting the acrylamide to brominated derivatives. These derivatives are described here in terms of their spectra and response in electron impact and positive chemical ionization. Additionally, a more direct and simple approach involving extraction and direct injection and analysis of acrylamide by positive chemical ionization is described. This screening approach is rapid, robust, and provides low detection limits.

Introduction

The discovery announced in April 2002 by scientists at Sweden's National Food Administration of acrylamide (2-propenamide) in fried and baked foods at levels many times that allowed in water suggested a much higher exposure than previously estimated [1-3]. Acrylamide (Figure 1), a known neurotoxin, is considered a probable human carcinogen. The World Health Organization considers 0.5 µg/L the maximum level for acrylamide in water. However, foods such as french fries, baked potato chips, crisp breads, and other common cooked foods, were found to contain acrylamide between 100 and 1000 µg/kg. Acrylamide was not found in the raw foodstuffs and cooking by boiling produced no detectable levels. Recent work has suggested that acrylamide forms via the Maillard reaction, which occurs when amino acids and sugars (for example, asparagine and sucrose) are heated together [4]. The concern over these relatively high concentrations has led to studies of the occurrence of acrylamide in a wide variety of foods.

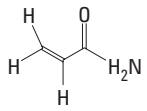


Figure 1 Acrylamide (2-propenamide), CH_2=CHCONH$_2$, 71.08 g/mole, CAS number 79-06-1.

Acrylamide Analytical Methodologies

A wide variety of instrumental approaches have been applied to acrylamide. Recent methods using

liquid chromatography with tandem mass spectrometry (MS-MS) detection have proved useful to approximately 50 µg/kg (ppb) or better using the 72 to 55 *m/z* transition (for example, [5]). This approach has appeared attractive in providing a simple sample preparation strategy. Gas chromatographic methods using MS detection with electron impact (EI) ionization typically suffer from the relatively small size of the molecule and therefore use derivatization. This application note presents alternative gas chromatography/ mass spectrometry (GC/MS) approaches aimed at more rapid screening, as well as the conventional, definitive quantitation via derivatization. These methods are rapid and relatively simple approaches to acrylamide analysis.

Rapid Screening via GC/MS-SIM with Positive Chemical Ionization

EI ionization mass spectrum for acrylamide (Figure 2) reveals very low mass ions; 71, 55, 44 *m/z*. Although there is good intensity at sub-ng levels, the ions are subject to interferences in food samples. The positive chemical ionization (PCI) spectrum achieved with ammonia provides more selective ionization and is of greater utility than EI in food matrices, Figure 3. Ammonia PCI results in two ions; 72 *m/z*, the protonated molecule, $[M+H]^+$, and 89 *m/z* due to the adduct, $[M+NH_4]^+$. PCI provides good selectivity and sensitivity for acrylamide–picogram amounts can be detected.

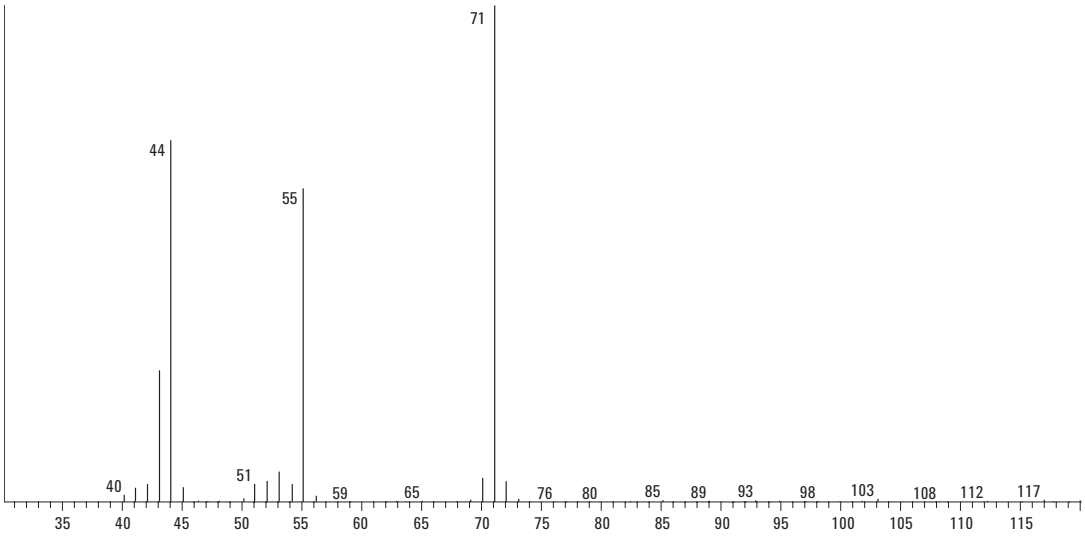


Figure 2 The EI ionization spectrum of acrylamide (40–120 amu).

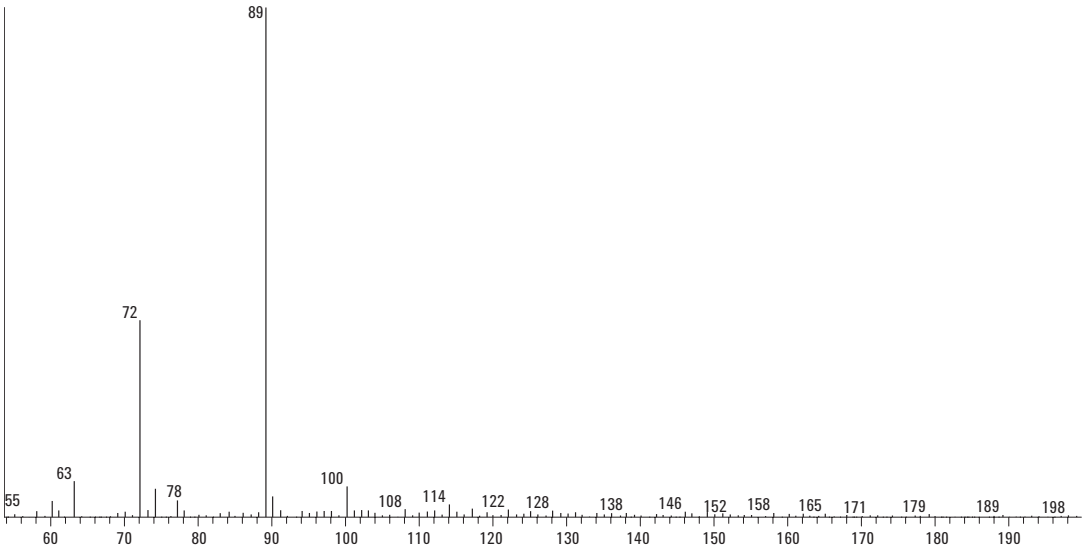


Figure 3 The PCI spectrum of acrylamide with ammonia reagent gas (60–200 amu).

Figure 4 shows a calibration curve from 100 pg to 10 ng collected under the method cited below in the section on Instrumental Parameters.

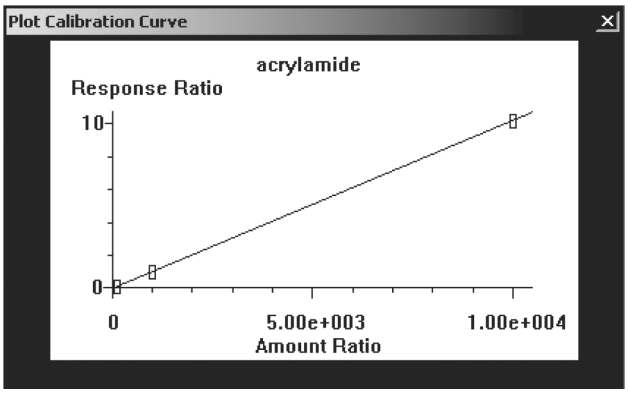


Figure 4 PCI-ammonia SIM calibration curve from 100-picograms to 10 ng ($R^2 = 1.00$).

Screening Sample Preparation

The enhanced specificity obtained through PCI can be used for rapid screening using a very simple and rapid sample cleanup. A food sample is homogenized and pulverized, and 0.4-g subsample is transferred to a centrifuge tube. The sample is extracted with 1 mL of methanol:water (9:1 v/v) solution for 10 minutes using an ultrasonic cleaner. Prior to sonication, 1 µg of labeled $^{13}C_3$-acrylamide is added to the 1-mL solution. After sonication, the sample is centrifuged for about 5 minutes at 8000 rpm. The upper layer is decanted and transferred to a vial for injection and analysis by GC/MS-PCI conditions with selected ion monitoring (SIM). See Table 1 for method parameters for PCI screening of native acrylamide.

Table 1. GC/MS Instrumental Method Parameters for PCI Screening of Native Acrylamide

Inlet parameters		
Liner:		Agilent p/n 5062-3587 Single-taper with glass wool
Temperature:		220 °C
Mode:		Pulsed splitless
Pulse pressure:		30.0 psi
Pulse time:		1.20 min
Purge flow:		50.0 mL/min
Purge time:		1.20 min
Total flow:		54.7 mL/min
Gas saver:		Off
Oven parameters		
Oven maximum:		260 °C
Oven equilibrium time:		0.20 min
Initial temperature:		60 °C
Initial time:		1.00 min
Ramp	Temperature	Time
12 °C/min	230 °C	10.00 min
Run time:		25.17 min
Column parameters		
Capillary column		Agilent 19091X-136 HP-INNOWax
Maximum temperature:		260 °C
Nominal length:		60.0 m
Nominal diameter:		250.00 µm
Nominal film thickness:		0.25 µm
Carrier:		Helium
Mode:		Constant flow 2.0 mL/min
Outlet and pressure:		MSD Vacuum
MSD Parameters		
Solvent delay		7.00 min
Tuning:		PCI Ammonia at 24% (1.2 mL/min)
EM Setting:		PCI Autotune + 400 V
Source temperature:		250 °C
Quad temperature:		150 °C
SIM Parameters		
Resolution:		High
Group ions		Dwell (ms)
72.0		60
75.0		60
89.1		60
92.1		60

Screening Method Results and Discussion

Figure 5 shows the extracted ion chromatograms for a sample of white bread. The baseline shows very little disturbance near the acrylamide analyte due to the selective nature of the PCI with ammonia. The extracted concentration is approximately 34 ng/mL or 85 ng acrylamide per gram white bread. Since acrylamide is formed when amino acids and sugars are heated together, it is logical to suspect the possibility of acrylamide formation in the inlet during injection. To test this possibility, the white bread extract was spiked with 100 ng of acrylamide and reanalyzed. The results calculated 135 ng/mL and suggest that either the relatively low temperature and short duration in the liner due to pressure pulsing mitigate acrylamide formation for this sample or acrylamide formed in the inlet is highly reproducible. This may not be the case in all extracts or under all similar conditions.

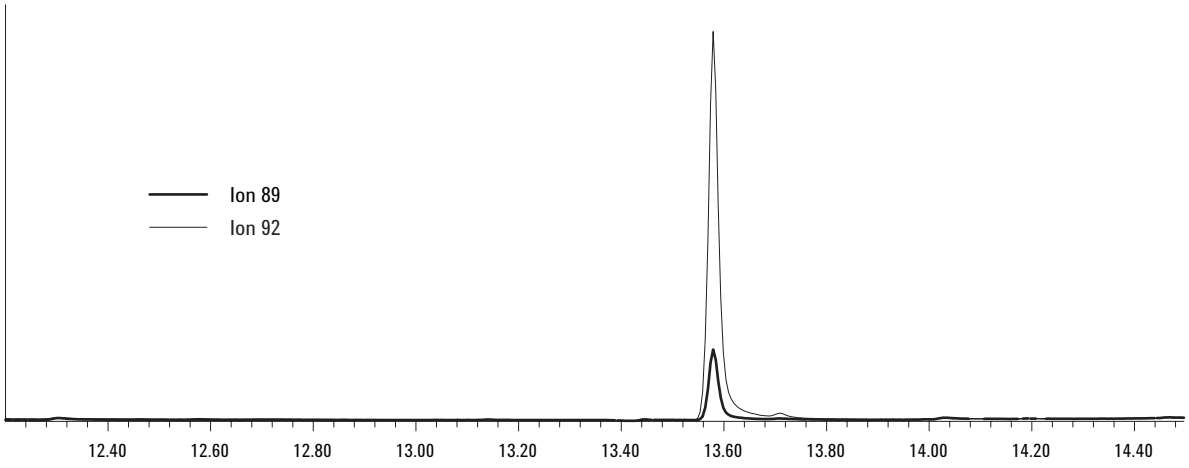
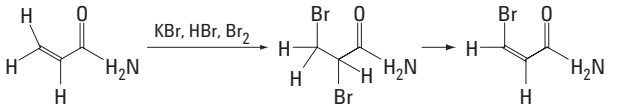


Figure 5. Extracted ion chromatograms for acrylamide (84 ng/g) in sample of white bread.

GC/MS Approaches to Acrylamide Involving Derivatives

Another approach to extraction from foods uses water, in situ derivatization, and liquid-liquid extraction [6, 7]. In this approach acrylamide in a homogenized sample is extracted with (hot) water, 1 g : 10 mL. A strong brominated agent is added and allowed to react. This reaction converts acrylamide to the 2,3-dibromopropionamide. Excess brominating reagent is removed by addition of sodium thiosulfate and the solution centrifuged and/or filtered. The 2,3-dibromopropionamide is extracted by partitioning into ethyl acetate. An option is to further treat this derivative to form a more stable analyte, the 2-bromopropenamide. The overall chemistry is given in Equation 1. Methacrylamide, CH_2=CH(CH_3)$CONH_2$, is frequently used as a recovery surrogate so its behavior is also reported here.



2,3-dibromopropionamide
$C_3H_5Br_2NO$
mol. wt. 230.9 g/mole

2-bromopropenamide
C_3H_4BrNO
mol. wt. 150.0 g/mole

Equation 1

Experimental

Acrylamide and methacrylamide were obtained as neat standards (Sigma-Aldrich Corp) and dissolved in HPLC grade methanol. Labeled acrylamide, 1,2,3-$^{13}C_3$-acrylamide, was obtained at 1 mg/mL methanol (Cambridge Isotope Laboratories, Andover, MA). The brominating reagent solution was made according to the literature [6] with reagent grade KBr, HBr, and bromine water (VWR, San Francisco,CA). Sodium thiosulfate was obtained as a 1-Normal solution (VWR, San Francisco,CA).

Derivatization also followed the literature [6] with addition of 1 mL of brominating reagent to solutions containing acrylamide; over-night derivatization, neutralization by 1-drop 1N sodium thiosulfate and extraction by 1-mL ethyl acetate (pesticide grade, VWR). The dibromo-derivatives were directly injected. The mono-bromo-derivatives were generated by addition of triethylamine.

Instrumental conditions for the dibromopropionamide and bromopropenamide are cited in Tables 2 and 3. All data was collected using 2-µL injections.

Table 2. GC/MS Instrumental Method Parameters for Dibromopropionamide (Dibromo-Derivative of Acrylamide) in EI and PCI with Methane and Ammonia

Inlet parameters		
Liner:		Agilent p/n5181-3315 double-taper
Temperature:		250 °C
Mode:		Pulsed splitless
Pulse pressure:		30.0 psi
Pulse time:		1.20 min
Purge flow:		50.0 mL/min
Purge time:		1.20 min
Total flow:		54.7 mL/min
Gas saver:		On
Oven parameters		
Oven maximum:		325 °C
Oven equilibrium time:		0.50 min
Initial temperature:		50 °C
Initial time:		1.00 min
Ramp	Temperature	Time
45 °C/min	300 °C	2.00 min
Run time:		8.56 min
Column parameters		
Capillary column		Agilent 122-3832 DB-35 ms
Maximum temperature:		340 °C
Nominal length:		30 m
Nominal diameter:		250 µm
Nominal film thickness:		0.25 µm
Carrier:		Helium
Mode:		Constant flow 1.2 mL/min
Outlet and pressure:		MSD Vacuum
MSD Parameters for EI and PCI		
Solvent delay		5.00 min
EI Parameters		
EI Tuning:		Autotune
EM Setting:		Autotune + 400 V
Source temperature:		230 °C
Quad temperature:		150 °C
EI SIM parameters		
Resolution:		Low

(Continued)

Table 2. GC/MS Instrumental Method Parameters for Dibromopropionamide (Dibromo-Derivative of Acrylamide) in EI and PCI with Methane and Ammonia (Continued)

Group ions	Dwell (ms)
2,3-dibromopropionamide	Acrylamide analyte
149.9	10 ms
151.9	10 ms
106.0	10 ms
$^{13}C_3$-2,3-dibromopropionamide	Internal standard
152.9	10 ms
154.9	10 ms
109.9	10 ms
2,3-dibromo-2-methylpropionamide	Ancillary surrogate
120.0	10 ms
122.0	10 ms
164.0	10 ms
166.0	10 ms
PCI Parameters	
PCI Tuning:	PCI Autotune
EM Setting:	PCI Autotune + 400 V
Source temperature:	250 °C
Quad temperature:	150 °C
PCI SIM Parameters	
Methane reagent gas:	MFC 20% (1.0 mL/min)
Resolution:	Low
Group ions	Dwell (ms)
2,3-dibromopropionamide	Acrylamide analyte
231.9	10 ms
233.9	10 ms
149.9	10 ms
151.9	10 ms
$^{13}C_3$-2,3-dibromopropionamide	Internal standard
234.9	10 ms
236.9	10 ms
2,3-dibromo-2-methylpropionamide	Ancillary surrogate
245.9	10 ms
247.9	10 ms
Ammonia reagent gas:	MFC 20% (1.0 mL/min)
Resolution:	Low
Group ions	Dwell (ms)
2,3-dibromopropionamide	Acrylamide analyte
248.9	10 ms
246.9	10 ms
250.9	10 ms
$^{13}C_3$-2,3-dibromopropionamide	Internal standard
251.9	10 ms
249.9	10 ms
253.9	10 ms
2,3-dibromo-2-methylpropionamide	Ancillary surrogate
262.9	10 ms
260.9	10 ms
264.9	10 ms

Table 3. GC/MS Instrumental Method Parameters for 2-bromopropenamide (Monobromo-Derivative of Acrylamide) in EI

Inlet parameters	
Liner:	Agilent p/n 5062-3587 Single-taper with glass wool
Temperature:	250 °C
Mode:	Pulsed splitless
Pulse pressure:	30.0 psi
Pulse time:	1.20 min
Purge flow:	50.0 mL/min
Purge time:	1.20 min
Total flow:	54.7 mL/min
Gas saver:	Off
Oven parameters	
Oven maximum:	325 °C
Oven equilibrium time:	0.50 min
Initial temperature:	50 °C
Initial time:	1.00 min
Column parameters	
Capillary column	Agilent 122-5533 DB-5MS

Ramp	Temperature	Time
25 °C/min	140 °C	0.00 min
45 °C/min	300 °C	1.50 min
Run time:		9.66 min

Maximum temperature:	350 °C
Nominal length:	30.0 m
Nominal diameter:	250 µm
Nominal film thickness:	1.00 µm
Carrier:	Helium
Mode:	Constant flow
1.2 mL/min	
Outlet and pressure:	MSD Vacuum
MSD Parameters for EI and PCI	
Solvent delay	5.00 min
EI Parameters	
EI Tuning:	Autotune
EM setting:	Autotune + 400V
Source temperature:	230 °C
Quad temperature:	150 °C
EI SIM Parameters	
Resolution:	Low
Group ions	Dwell (ms)
2-bromopropenamide	Native acrylamide
148.9	20 ms
150.9	20 ms
105.9	20 ms
$^{13}C_3$-2-bromopropenamide	Internal standard
151.95	20 ms
153.95	20 ms
2,3-dibromo-2-methylpropionamide	Ancillary surrogate
120.0	10 ms
122.0	10 ms
164.0	10 ms
166.0	10 ms

Results and Discussion

EI Ionization

Figures 6 and 7 show the EI mass spectrum of the 2,3-dibromopropionamide and the 2-bromopropenamide, respectively. Note the similar spectra for the two brominated acrylamide derivatives. In EI, the 2,3-dibromopropionamide loses bromide to generate the C_3H_5ONBr ion that shows the isotopic abundance expected from a monobrominated species. The addition of the triethylamine (base) leads to loss of HBr in solution, generating the monobrominated species C_3H_4ONBr which contains one less hydrogen than the dibromo-derivative and appears as the molecular ion in EI. The spectra share a common C_2H_3Br ion that accounts for the fragments at 105.9 and 107.9 *m/z*. Note that use of the $^{13}C_3$-acrylamide as an internal standard prohibits use of the 107.9 ion in acrylamide quantitation due this C_2H_3Br fragment. The dibromo-derivative shows greater response than the monobrominated compound and lacks the 149 fragment which is subject to interferences from phthalates which are ubiquitous in solvents and food packaging. Both compounds demonstrate good linearity over the range of 10 to 500 pg/µL in EI-SIM as shown in Figures 8 and 9, but better EI detection and elution at a higher oven temperature makes the dibromo-derivative more attractive than the monobromo-derivative. However, it has long been known that the 2,3-dibromopropionamide breaks down in the injection port to form the 2-bromopropenamide. The fraction converted is a function of the injection port activity hence the use of the double-tapered liner for the dibromopropionamide analysis as opposed to the single-tapered liner with wool for the bromopropenamide. Use of the ^{13}C-labeled surrogate is necessary to correct for the degradation of the dibromo-derivative but the methacrylamide surrogate may correct fairly well for recoveries of the mono-brominated acrylamide. Because of this and citations of its use in the literature, the EI spectrum for the brominated methacrylamide is shown in Figure 10 and ions are presented in the acquisition method tables. As the 2,3-dibromo-2-methylpropionamide, this surrogate elutes just prior to the 2,3-dibromopropioamide and much later than the 2-brompropenamide on the GC programs cited.

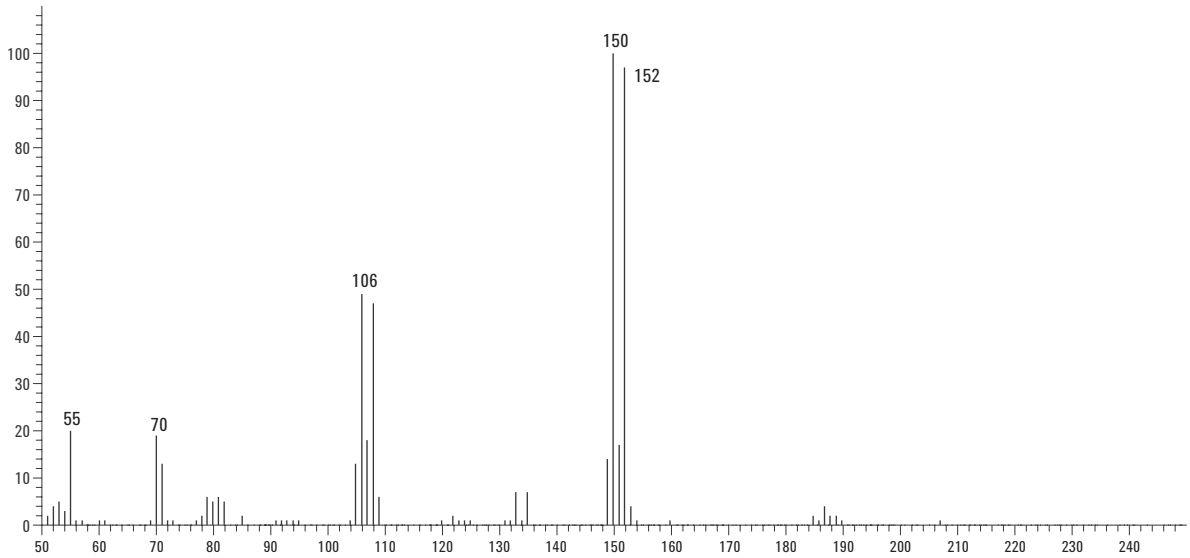


Figure 6. EI ionization spectrum of 2,3-dibromopropionamide.

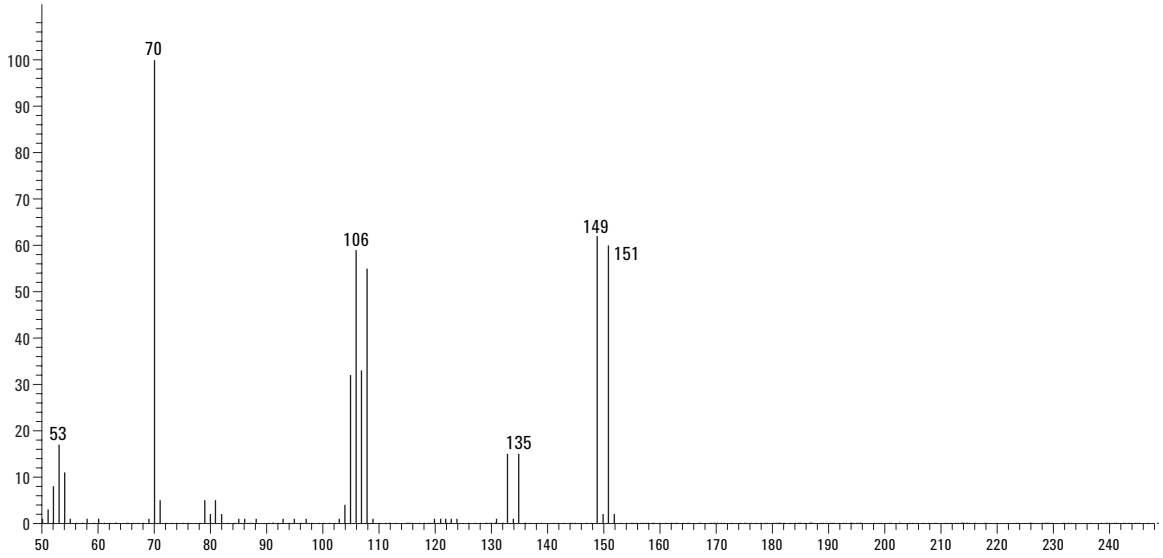


Figure 7. EI ionization spectrum of 2-bromopropenamide.

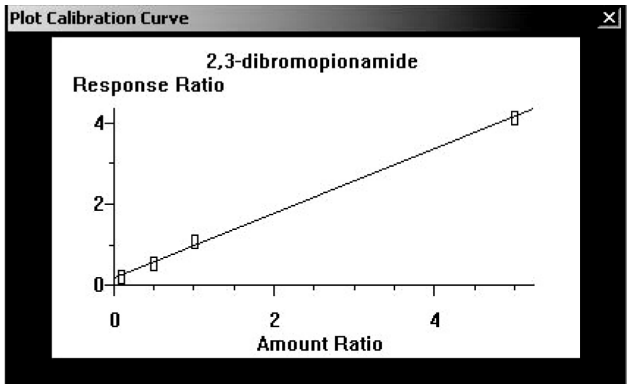


Figure 8. Calibration Curve plot for 2,3-dibromopropionamide from 10 to 500 pg/µL ($R^2 = 0.998$).

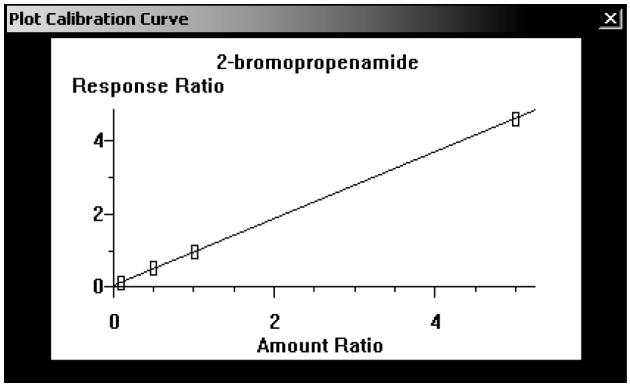


Figure 9. Calibration Curve plot for 2-bromopropenamide from 10 to 500 pg/µL ($R^2 = 0.999$).

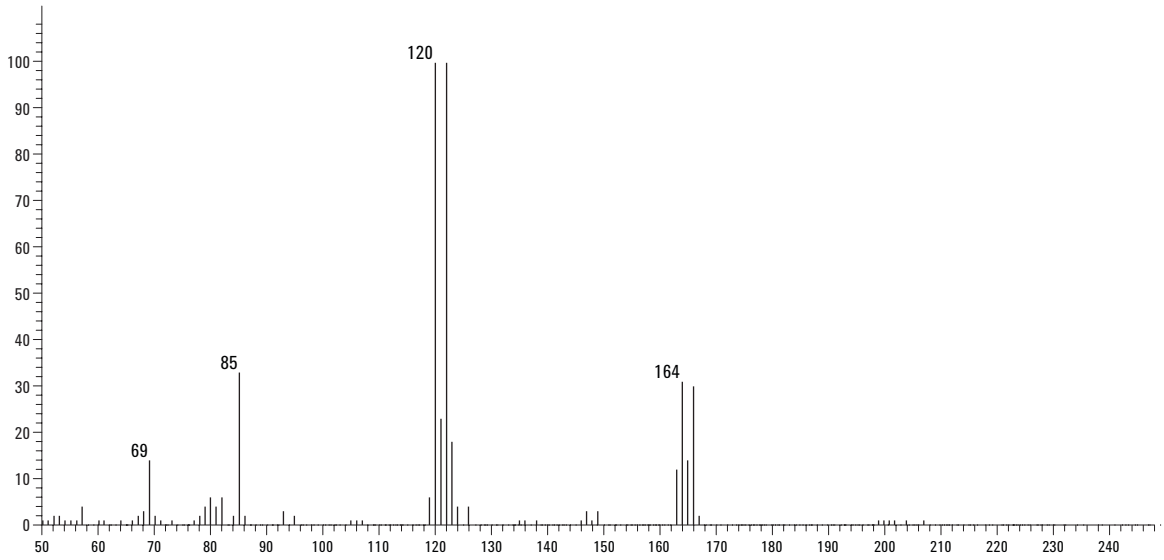


Figure 10. EI ionization spectrum of the alternative, methacrylamide surrogate, 2,3-dibromo-2-methylpropionamide.

PCI

The 2,3-dibromopropionamide spectra in PCI with methane and ammonia reagent gas are shown in Figures 11 and 12. In methane, the highest mass fragment is due to $[M+H]^+$ and in ammonia, $[M+NH_4]^+$. Response with methane is higher than with ammonia and would make a good choice in acrylamide quantitation in samples, if background for that particular food are not an issue. Calibration is similar to that in EI between 10 and 500 pg/µL for both methane and ammonia (R^2 >0.998). It is important that the lower mass fragments that occur in methane and ammonia PCI, *m/z* 72 and 89, respectively, are not used in SIM quantitation. These intense fragments apparently originate through elimination of Br_2 and do not coincide with the cited ions.

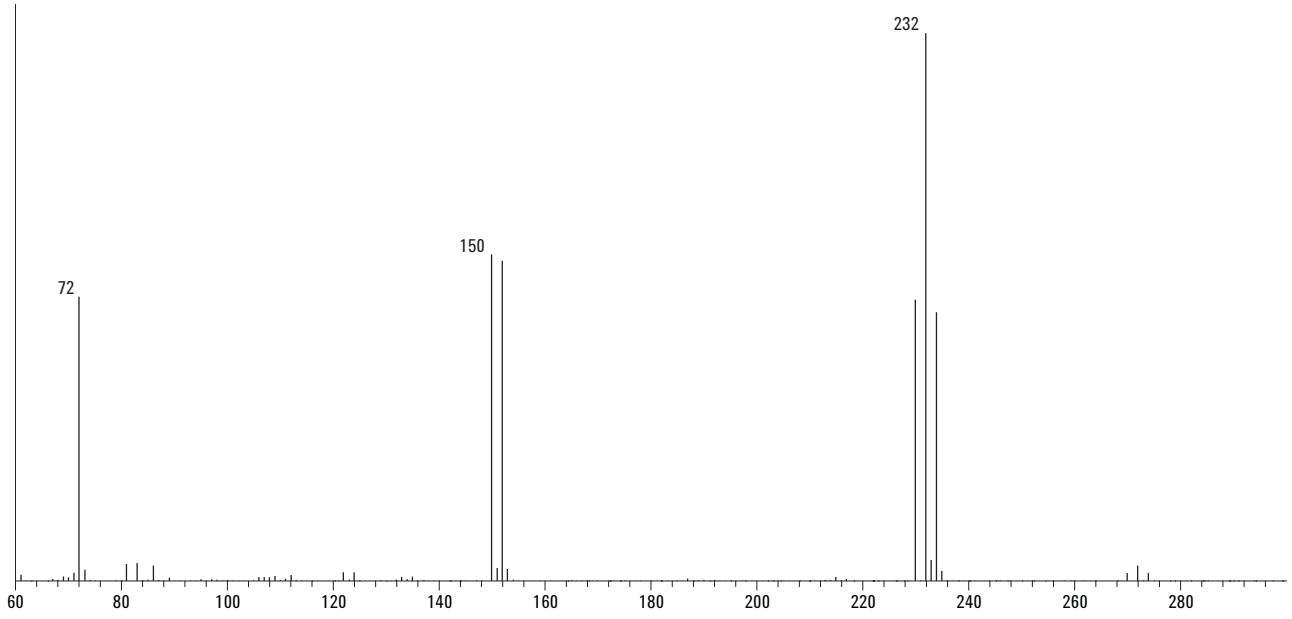


Figure 11. The PCI spectrum of 2,3-dibromopropionamide with methane reagent gas (60–300 amu).

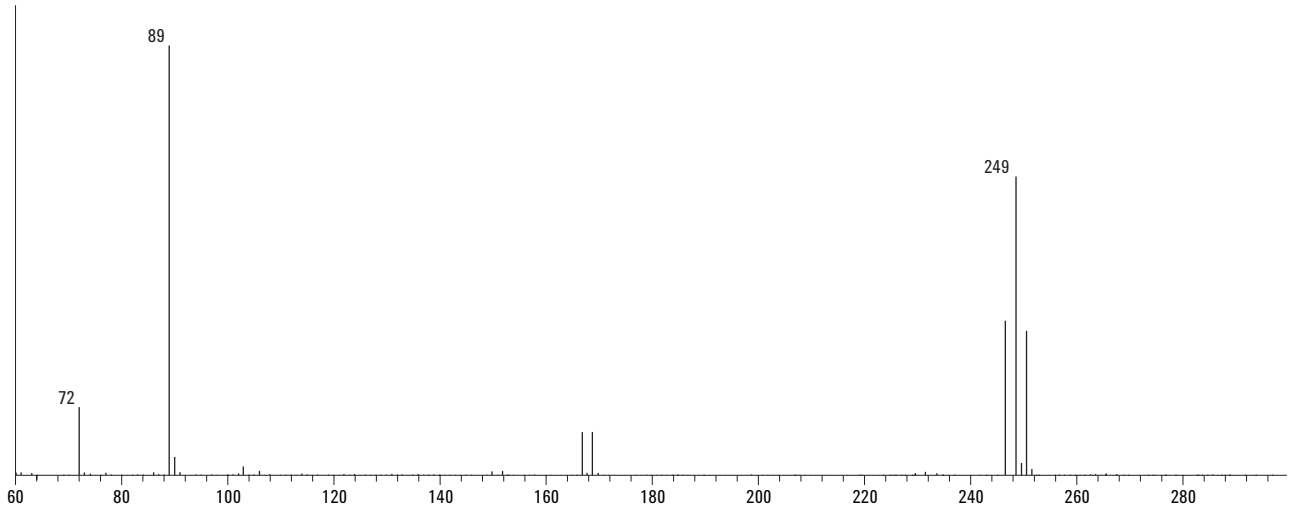


Figure 12. The PCI spectrum of 2,3-dibromopropionamide with ammonia reagent gas (60–300 amu).

Similar to the situation in EI, PCI response of the 2,3-dibromopropionamide exceeds that of the 2-bromopropenamide under either reagent gas. Spectra for this analyte using methane and ammonia are presented in Figures 13 and 14. Highest mass fragments for 2-bromopropenamide also are due to $[M+H]^+$ in methane and in ammonia, $[M+NH_4]^+$. For completeness, the spectra are also included for the brominated methacrylamide surrogate, Figure 15 and 16.

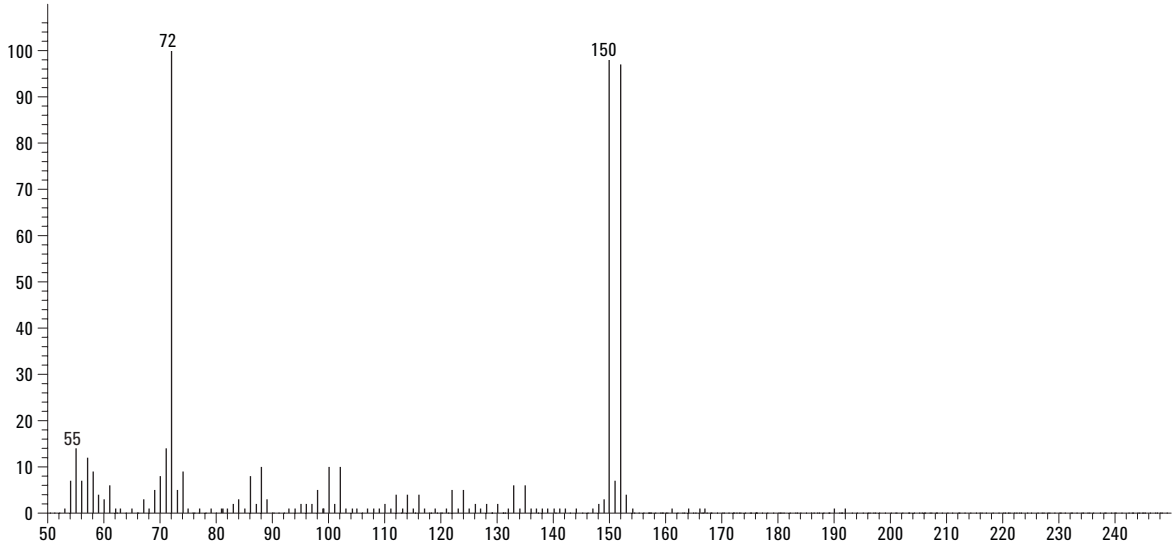


Figure. 13 The PCI spectrum of 2-bromopropenamide with methane reagent gas (50–250 amu).

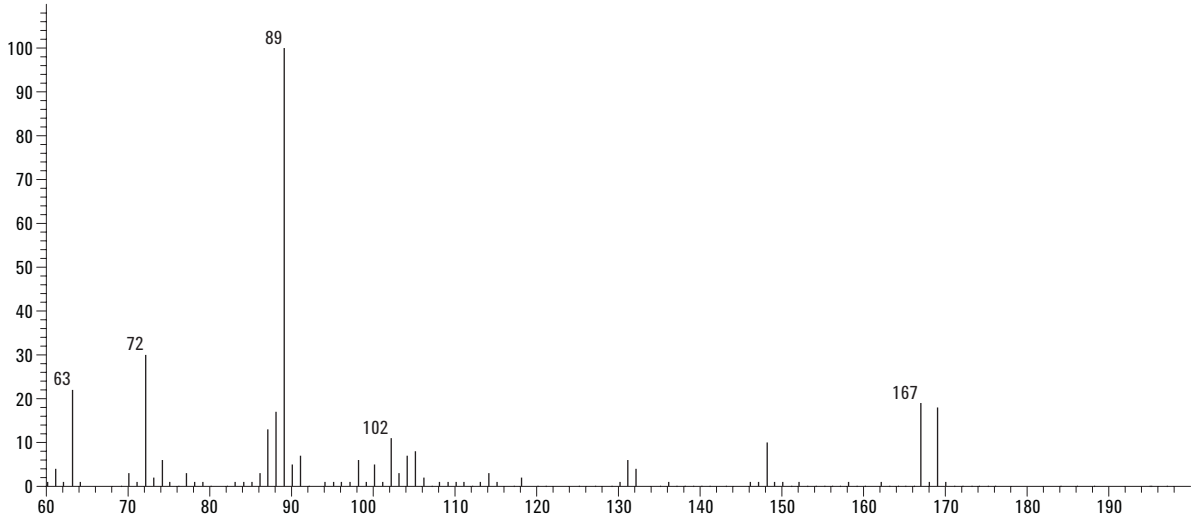


Figure 14. The PCI spectrum of 2-bromopropenamide with ammonia reagent gas (60–200 amu).

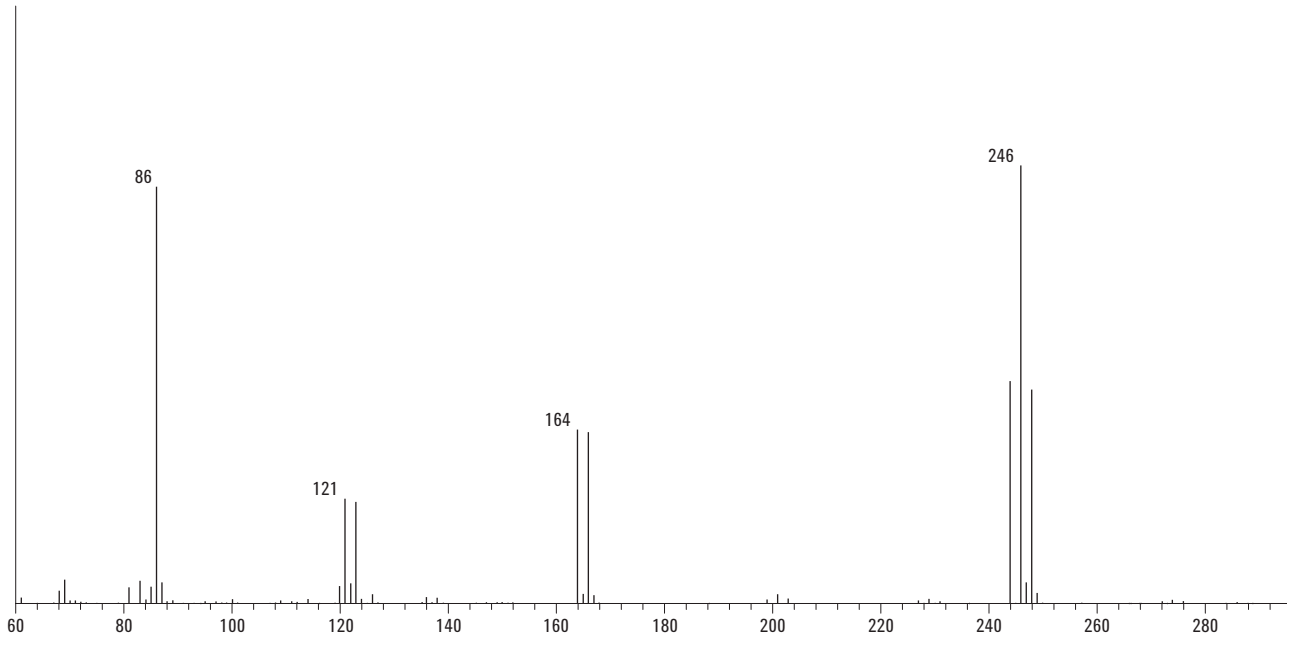


Figure 15. The PCI spectrum of 2,3-dibromo-2-methylpropionamide (the methacrylamide derivative) with methane reagent gas (60–300 amu).

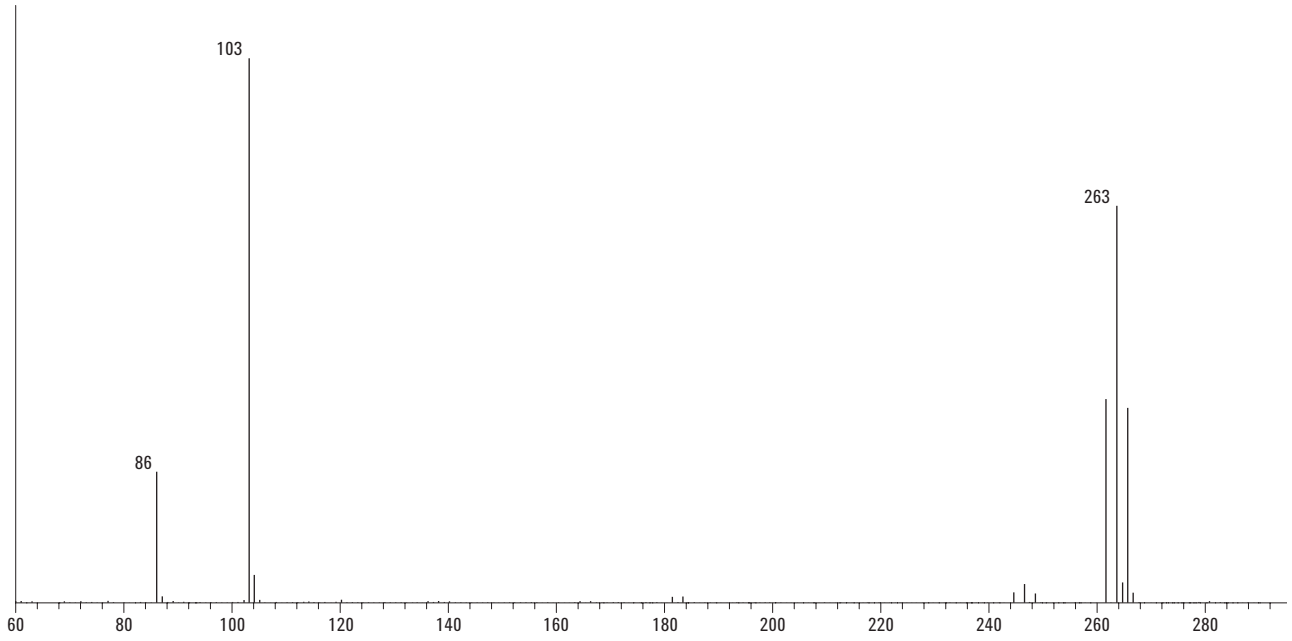


Figure 16. The PCI spectrum of 2,3-dibromo-2-methylpropionamide with ammonia reagent gas.

Conclusions

Since acrylamide was found in a wide range of foodstuffs, a variety of approaches were presented here. The rapid screening approach for native acrylamide using PCI provides a direct and simple method for sensitive detection and quantitation. For approaches using the brominated derivatives, the dibromopropionamide shows superior opportunities for detection and quantitation relative to the 2-bromopropenamide. If, for a particular food product, there are problems in EI, PCI will provide a worthwhile approach for exploration. Methane reagent gas provides about twice the response of ammonia. The degradation of the dibromopropionamide can and must be accounted for by an appropriate labeled internal standard. The methacrylamide surrogate also may be useful for recovery calculations. Data collected on potato chips, and not presented here, suggests this is the case.

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