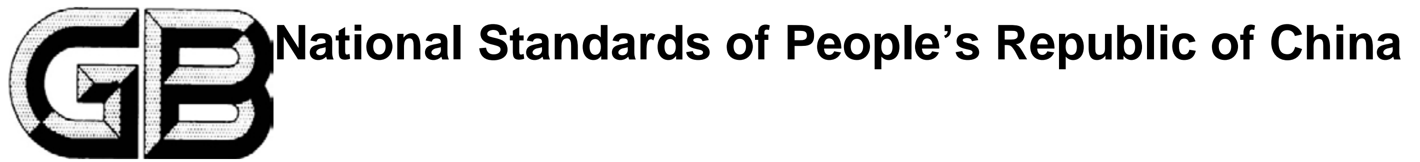

National Standards of People's Republic of China

GB 5009.204-2014

National Food Safety Standard

Determination of Acrylamide in Foods

Issued on: 2014-12-01 Implemented on: 2015-05-01

Issued by National Health and Family Planning Commission

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Foreword

This standard has substituted for GB/T 5009.204—2005 Determination of Acrylamide in Food with the GC-MS method.

Compared with GB/T 5009.204—2005, this standard has the following main changes:

- Method I, Mass-spectrography, gas chromatography to dilute stable isotopes, has been added.
- The GC-MS method has changed the external standard method into stable isotopes dilution method.

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National Standard for Food Safety

Determination of Acrylamide in Foods

1. Scope

The standard specifies the method to detect acrylamide in foods.

The standard is applicable to the determination of acrylamide in heat processed (fried, baked, roasted, grilled, etc) foods.

Method I Liquid chromatography-mass spectrography/ mass-spectrography to dilute stable isotopes

2. Principles

The stable isotope dilution technique is applied in the standard to add 13C12-marked PCBs into the samples with water as the extraction solvent. After the solid phase extraction or base material solid phase extraction purification, detection shall be made with the multiple reactions monitoring (MRM) of Liquid chromatography-mass spectrography/ mass-spectrography or selective reaction monitoring (SRM) and determination with the internal standard method shall be carried out.

3. Reagents and materials

Notes: Unless otherwise stated, all reagents in the method shall be analytically pure and the water shall be Grade I water specified in GB/T 6682.

3.1 Reagents

3.1.1 Methanoic acid (HCOOH): chromatographically pure

3.1.2 Methyl alcohol (CH_3OH): chromatographically pure

3.1.3 Normal hexane (n-C_6H_{14}): analytically pure, used after being re-distilled.

3.1.4 Ethyl acetate ($CH_3COOC_2H_5$): analytically pure, used after being re-distilled.

3.1.5 Anhydrous Na_2SO_4: baked at 400 ℃ for 4 hours.

3.1.6 Ammonium sulfate [$(NH_4)_2SO_4$].

3.1.7 Diatomite: ExtrelutTM 20 or equivalent products.

3.2 Standard products

3.2.1 Acrylamide (CH2=CHCONH2) standard products (purity>99%)

3.2.2 3.2.2 13C3-Acrylamide13CH2=13CH13CONH2) standard products (purity>98%)

3.3 Formulation of standard solution

3.3.1 Formulation of acrylamide standard solution

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3.3.1.1 Acrylamide standard stock solution(1,000 mg/L):accurately weigh acrylamide standard products and dissolve it with methanol to make the acrylamide concentration reach 1,000 mg/L before storing it in the refrigerator at -20 ℃

3.3.1.2 Acrylamide midst solution(100 mg/L): Transfer 1 mL of acrylamide standard stock solution and dilute it to 10 mL with methanol to make the acrylamide concentration reach 100 mg/L before storing it in the refrigerator at -20 ℃

3.3.1.3 Acrylamide work solution I (10 mg/L): Transfer 1 mL of acrylamide midst solution and dilute it to 10 mL with 0.1% methanoic acid solution to make the acrylamide concentration reach 10 mg/L. It shall be formulated when being used.

3.3.1.4 Acrylamide work solution II (1 mg/L): Transfer 1 mL of acrylamide work solution I and dilute it to 10 mL with 0.1% methanoic acid solution to make the acrylamide concentration reach 1mg/L. It shall be formulated when being used.

3.3.2 $^{13}C_3$-acrylamide internal standard solution

3.3.2.1 $^{13}C_3$-acrylamide internal standard stock solution(1,000 mg/L):accurately weigh $^{13}C_3$-acrylamide standard product and dissolve it with methanol to make the $^{13}C_3$-acrylamide concentration reach 1,000 mg/L before storing it in the refrigerator at -20 ℃

3.3.2.2 Internal standard work solution(10 mg/L):Transfer 1 mL of internal standard stock solution and dissolve it to 100 mL with methanoic acid to make the $^{13}C_3$-acrylamide concentration reach 10 mg/L before storing it in the refrigerator at -20 ℃

3.3.3 Standard curve work solution

Take 6 10-mL volumetric flasks to separately transfer 0.1 mL, 0.5 mL and 1 mL of acrylamide work solution II (1 mg/L), 1 mL and 3 mL of acrylamide work solution I (10 mg/L) and 0.1 mL of internal standard work solution (10 mg/L). Then dilute it with 0.1% methanoic acid solution to the scale. Acrylamide concentration in the standard solution shall be separately 10 μg/L, 50 μg/L, 100 μg/L, 500 μg/L, 1,000 μg/L and 3,000 μg/L. The internal standard concentration shall be 100 μg/L. It shall be formulated when being used.

4. Equipment and facilities

4.1 Liquid chromatogram-mass spectrum/mass spectrometer (LC-MS/MS)

4.2 HLB solid phase extraction column:6 mL, 200 mg, or equivalent products

4.3 Bond Elut-Accucat solid phase extraction column:3 mL, 200 mg，or equivalent products

4.4 Tissue grinder

4.5 Rotary evaporator

4.6 Nitrogen inspissator

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4.7 Oscillator

4.8 Glass chromatographic column: column length--30 cm, column internal diameter --1.8 cm

4.9 Turbine mixer

4.10 Ultra-pure water equipment

4.11 Analytical balance whose sensitivity is 0.1 mg

4.12 Centrifuge: rotate speed≤10,000r/m.

5. Analytical procedure

5.1 Specimen preparation

5.1.1 Sample extraction

Take 50 g of samples and grind them with the grinder and then store the at -20℃Accurately weigh 1 g to 2 g of samples (correct to 0.001 g). 10 μL or 20 μL of $^{13}C_3$-acrylamide internal standard (10 mg/L) shall be added, which is equal to 100 ng or 200 ng of $^{13}C_3$-acrylamide internal standard. Then add 10 mL of ultra-pure water and shake it for 30 minutes to centrifuge it at 4,000 r/m for 10 minutes. Finally acquire the liquid supernatant for purification.

5.1.2 Sample purification

Notes: any of the following methods shall be chosen for purification.

5.1.2.1 Base material solid-phase dispersion extraction method (Selection 1):add 15 g of ammonium sulfate into the liquid supernatant extracted from the samples and shake for 10 minutes to fully dissolve it before centrifuging it at 4,000 r/m for 10 minutes. Get 10 mL of liquid supernatant for use. When the liquid supernatant doesn't reach 10 mL, saturated ammonium sulfate shall be used for complement. Put a little glass wool in the bottom of a pure glass chromatography column and press it tightly. Then fill in 10 g of anhydrous Na_2SO_4 and 2g of diatomite in sequence. Mix 5 g of diatomite Extrelut™ 20 with the liquid supernatant of the above samples and then fill it in the chromatographic column. Rinse it with 70 mL of normal hexane at 2 mL/min and then remove the normal hexane. Elute the acrylamide with 70mL of ethyl acetate at 2 mL/min. Then collect the ethyl acetate elution solution and place it under the vacuum-rotary evaporation procedure at the 45 ℃water bath until it's nearly dry. Rinse the residue in the evaporating flask with ethyl acetate for three times (1 mL every time) and then transfer it into the test tube which already has 1 mL of 0.1% methanoic acid solution before vortex oscillation. Blow away the upper organic phase with nitrogen and add in 1 mL of normal hexane before vortex oscillation. Then centrifuge it at 3,500 r/m for 5 minutes. Filter the upper organic phase with the 0.22 μm aqueous phase filter membrane for LC-MS/MS test.

5.1.2.2 Solid phase extraction column(Selection 1I): Add 5 mL of normal hexane into the liquid supernatant from the samples for a 10-minute oscillation extraction. Then centrifuge it at 10,000 r/m for 5 minutes. Remove the organic phase and extract it again with 5 mL of normal hexane. Then rapidly get 6 mL of aqueous phase and filter it with the 0.45-μm aqueous phase filter and carry out HLB solid phase extraction column purification. Activate the HLB solid phase extraction column with 3 mL of methyl alcohol and 3 mL of water. Apply 5 mL of the above filter liquor to the HLB solid phase extraction column and

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collect the effluent. Then eluate it with 4 mL of 80% methanol water fluid and collect all the eluate. Then mix it with the effluent for Bond Elut-Accucat solid phase extraction column purification; Activate the Bond Elut-Accucat solid phase extraction column with 3 mL of methyl alcohol and 3mL of water. Add all the samples of eluate for the HLB solid phase extraction column purification and make it outflow under the action of gravity. Then collect all the eluate and concentrate it under the nitrogen flow until it's nearly dry. Finally dilute the 0.1% methanoic acid solution to 1.0 mL for LC-MS/MS determination.

5.2 Reference conditions for instruments

5.2.1 Chromatographic conditions

The chromatographic column shall be Atlantis C_{18} Column (5 μm, 2.1 mm I.D.×150 mm) or equivalent columns.

Pre-column: C18 Guard Column (5 μm, 2.1 mm I.D.×30 mm) or equivalent columns.

Mobile phase: methyl alcohol/0.1% methanoic acid(10:90，volume fraction)

Flow velocity:0.2 mL/min

Sample injection volume: 25 μL

Column temperature: 26 ℃

5.2.2 Mass spectrometric parameters

5.2.2.1 Triple quadrupole tandem mass spectrometer

Detection mode:multiple reaction monitoring(MRM)

Ionization mode:ESI+

Capillary voltage:3 500 V

Cone voltage:40 V

Radio-frequency lens 1 voltage: 30.8 V

Ion source temperature: 80 ℃

Desolvation Temperature: 300 ℃

Ion collision energy: 6 eV

Acrylamide:parent ion m/z 72, daughter ion m/z 55, daughter ion m/z 44

$^{13}C_3$ acrylamide:parent ion m/z 75, daughter ion m/z 58, daughter ion m/z 45

Quantitative ion: acrylamide, m/z 55

$^{13}C_3$ acrylamide:m/z 58

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5.2.2.2 Ion trap tandem mass spectrometer

Detection mode: SRM

Ionization mode: ESI+

Spray voltage: 5,000 V

Heating capillary temperature: 300 ℃

Sheath gas:N_2，40 Arb

Auxiliary gas:N_2，20 Arb

Collision induced dissociation (CID):10 V

Collision Energy: 40 V

Acrylamide: parent ion m/z 72, daughter ion m/z 55, daughter ion m/z 44

$^{13}C_3$ acrylamide: parent ion m/z 75, daughter ion m/z 58, daughter ion m/z 45

Quantitative ion: acrylamide, m/z 55；

$^{13}C_3$ acrylamide: m/z 58

5.3 Mapping of standard curves

Infuse the standard work liquid into Liquid Chromatogram-Mass Spectrum/ Mass Spectrometry System to detect the peak area of corresponding acrylamide and their internal standard. Standard curves shall be mapped by taking the acrylamide sampling concentration of all standard work liquid(μg/L)as the abscissa and the peak area of acrylamide(m/z 55)and $^{13}C_3$ acrylamide internal standard (m/z 58) as the ordinate.

5.4 Determination of the sample solution

Infuse the sample solution into the Liquid Chromatogram-Mass Spectrum/ Mass Spectrometry System and measure the peak area ratio of acrylamide(m/z 55)and $^{13}C_3$ acrylamide internal standard (m/z 58). Acrylamide sampling concentration (μg/L) in the to-be-tested liquid is acquired according to standard curves. Parallel determination shall be done twice or more.

5.5 Mass spectrometry

Infuse the samples and standard work liquid into the Liquid Chromatogram- Mass Spectrum/Mass Spectrometer in sequence to record the total ion chromatogram and mass spectrum (see Fig A.1 to A.2 at Appendix A)， as well as the peak area of acrylamide and internal standard and keep the abundance

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qualification of time and fragment ions. The chromatographic peak SNR of detected acrylamide shall be more than 3. The retention time of target compounds in detected samples shall be in accordance with that in the standard solution. The abundance ratio of the relevant monitoring ions of the target compounds in tested samples shall be in accordance with that in the standard solution. See the permissible deviation at Table 1.

Table 1 The maximum permissible deviation of relevant ion abundance in qualitative tests

Relevant ion abundance (base line peak	Permissible relative deviation
＞50%	±20%
＞20%～50%	±25%
＞10%～20%	±30%
≤10%	±50%

6. Expression of the analysis results

The acrylamide content in the samples shall be calculated according to the Formula (1)Internal Standard Method:

$$X = \frac{A \times f}{M} \quad \cdots\cdots (1)$$

In the formula:

X—acrylamide content in samples (μg/kg)

A—acrylamide weight which is in accordance with the peak area ratio of acrylamide(m/z 55)chromatographic peak and 13C3 acrylamide internal standard (m/z 58) chromatographic peak in the samples (ng)

f—conversion factors of internal standard adding quantity in the samples (f=1 when the internal standard is 10 μL or f=2 when the internal standard is 20 μL)

M—Sample volume added into the internal standard (g)

Calculation results shall be shown with the arithmetic mean value of two individually analysis results acquired on a repetitive basis. The outcome shall be calculate to a three-effective-digit number (or to the first decimal place)

7. Precision

The absolute difference of the two independent analysis results acquired on a repetitive basis shall not exceed the 20% of the arithmetic mean value.

8. Others

Limit of quantitation is 10 μg/kg.

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Method II Gas chromatography-mass spectrography to dilute stable isotopes

9. Principles

The standard shall adopt the stable isotope dilution technique and add 13C3-marked acrylamide internal standard solution into the samples. By taking water as the extraction solvent , the sample extracting solution makes a detection with MRM or SIM after adopting base material solid-phase dispersion extraction purification and bromide reagent derivation.Determination with the internal standard method shall be used.

10. Reagents and materials

Notes: Unless otherwise stated, all reagents in the method shall be analytically pure and the water shall be ultra-pure water.

10.1 Reagents

10.1.1 Normal hexane (n-C_6H_{14}):analytically pure, used after being re-distilled.

10.1.2 Ethyl acetate ($CH_3COOC_2H_5$):analytically pure, used after being re-distilled.

10.1.3 Anhydrous Na_2SO_4: baked at 400 ℃for 4 hours.

10.1.4 Ammonium sulfate [$(NH_4)_2SO_4$].

10.1.5 Sodium thiosulfate($Na_2S_2O_3.5H_2O$).

10.1.6 Bromine (Br_2).

10.1.7 Hydrobromic acid: content>48.0%.

10.1.8 Potassium bromide (KBr).

10.1.9 Ultra-pure water, conductivity (25℃)≤0.01 mS/m.

10.1.10 Bromizating agent.

10.1.11 Diatomite: Extrelut™ 20 or equivalent products.

10.2 Reagent compounding

10.2.1 Saturated bromine water:Measure 100 mL of ultra-pure water and place them in the 200-mL brown reagent bottle. Then add in 8 mL of bromine and keep it out of the sun at 4 ℃for 8 hours. The upper level is saturated bromine solution.

10.2.2 Bromizating agent: Weigh 20.0 g of potassium bromide and add 50 mL of ultra-pure water to totally dissolve it. Then add in 1.0 mL of hydrobromic acid and 16.0 mL of saturated bromine water. Shake it up and dilute it to 100 mL with ultra-pure water. Keep it out of the sun at 4 ℃

10.2.3 Sodium thiosulfate solution (0.1 mol/L): Weigh 2.48 g of sodium thiosulfate and add in 50 mL of ultra-pure water to totally dissolve it. The dilute it to 100 mL with ultra-pure water and keep it out of the sun at

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4 ℃

10.2.4 Saturated ammonium sulfate solution: Weigh 80 g of ammonium sulfate crystals and add in 100 mL of ultra-pure water. Dissolve it with the ultrasound and place it at the room temperature.

10.3 Standard products

10.3.1 Acrylamide (CH2=CHCONH2) standard products: purity>99%

10.3.2 13C 3 -acrylamide(13CH2=13CH13CONH2)standard products: purity>98%

10.4 Formulation of standard solutions

10.4.1 Acrylamide and its internal solution: alike to 3.3.1 and 3.3.2.

10.4.2 Standard curve work solution: Take 5 10-mL volumetric flasks to separately transfer 0.1 mL, 0.5 mL and 2 mL of acrylamide work solution Ⅱ(1 mg/L) and 0.5 mL and 1 mL of crylamide work solution Ⅰ(1 mg/L), as well as 0.5 mL of internal standard work solution(1 mg/L), with 5 10-mL volumetric flasks. The acrylamide concentration in the standard solution shall be 10 μg/L, 50 μg/L, 200 μg/L, 500 μg/L and 1,000 μg/L. The internal concentration shall be 50 μg/L. It shall be formulated when being used.

11 Equipment and facilities

11.1 Gas chromatograph-quadrupole mass spectrometer(GC-MS)

11.2 Chromatographic column:DB-5ms column (30 m×0.25 mm×0.25 μm) or equivalent chromatographic column

11.3 Tissue grinder

11.4 Rotary evaporator

11.5 Nitrogen concentrator

11.6 Oscillator

11.7 Glass chromatographic column: column length-30 cm, column internal diameter -1.8cm

11.8 Turbine mixer

11.9 Ultra-pure water equipment

11.10 Analytical balance whose sensitivity is 0.1 mg

11.11 Centrifugal machine: revolving speed≤10,000 r/m

12 Analytical procedures

12.1 Specimen preparation

12.1.1 Sample extraction

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Grind 50 g of the samples with the grinder and store it at -20℃. Accurately weigh 2 g of samples (correct to 0.001 g). Add 10 μL or 20 μL of 13C3-acrylamide internal standard solution (10.0 mg/L), which is equal to 100 ng or 200 ng of 13C3-acrylamide internal standard solution. Shake it for 30 minutes and centrifuge it at 4,000 r/m for 10 minutes. Collect the liquid supernatant for use.

12.1.2 Sample purification

Add 15 g of ammonium sulfate to the liquid supernatant extracted from the samples and shake it for 10 minutes to fully dissolve it. Centrifuge it at 4,000 r/m for 10 minutes. Collect 10 mL of liquid supernatant for use. Complement the liquid supernatant with saturated ammonium sulfate when the liquid supernatant is less than 10 mL. Fill a little glass wool in the bottom of a clean glass chromatographic column and press it tight. Then fill in 10 g of anhydrous Na2SO4 and 2 g of ExtrelutTM 20 diatomite.Weigh 5g of ExtrelutTM 20 diatomite and evenly mix it with the above prepared liquid supernatant samples before filling it in the chromatographic column. Rinse it with 70 mL of normal hexane and keep the flow velocity at 2 mL/min. Then remove the normal hexane eluent. Elute it with 70 mL of ethyl acetate and keep the flow velocity at 2 mL/min. Then collect the ethyl acetate elution and nearly dry it under decompression and rotary evaporation in the 45 ℃ water bath. Rinse the residues in the evaporating flasks with ethyl acetate for three times (1 mL every time). Transfer it to the test tube with 1 mL of ultra-pure water for vortex oscillation. Blow away the upper organic phase in the nitrogen flow and add 1 mL of normal hexane for vortex oscillation. Then centrifuge it at 3,500 r/m for 5 minutes and collect the lower aqueous phase for derivation.

12.1.3 Derivation

Sample derivation: add 1 mL of bromizating agent into the sample extracting solution for vortex oscillation. Place it at 4 ℃ for at least 1 hour and add in approximately 100 μL of sodium thiosulfate solution (0.1 mol/L) for vortex oscillation to remove the residual derivative agents. Add in 2 mL of ethyl acetate for vortex oscillation for 1 minute. Centrifuge it at 4,000 r/m for 5 minutes. Transfer the upper organic phase to the test tube which contains 0.1 g of anhydrous sodium sulfate. Then repeat the extraction with 2 mL of ethyl acetate and combine the organic phase. Still it for at least half an hour and transfer it to the other test tube before blowing it until it's nearly dry.Then add in 0.5 mL of ethyl acetate to dissolve the residue and leave it for use (Notes: the volume of ethyl acetate which is used to dissolve the residues shall be adjusted according to the sensitivity of instruments. Usually, its dosage shall be 0.5 mL when tandem mass spectrometers are used for detection and 0.1 mL when monopole mass spectrometers are used).

Derivation of standard solution: measure several units of 1.0 mL standard solution and synchronously carry out the above sample derivation method.

13 Reference conditions for instruments

13.1 Chromatographic conditions

Chromatographic column: DB-5ms column (30 m×0.25 mm I.D×0.25 μm) or equivalent chromatographic column

Injection port temperature: remain at 120 ℃ for 2 minutes and then rise to 240 ℃ at 40 ℃/min where it stays for 5 minutes.

Chromatographic column program temperature: remain at 65 ℃ for 1 minute and then rise to 200 ℃ at 15 ℃/min and then rise to 240 ℃ at 40 ℃/min where it stays for 5 minutes.

Carrier gas: high-purity helium (purity>99.999%), Pre-column pressure-69 mPa, equal to 10 psi.

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Unsplit stream sampling: injection volume-1 μL

13.2 Mass spectrometric parameters

Detection mode: SIM collection

Ionization mode: Electron Impact (EI), energy 70 eV

Transmission line temperature: 250 ℃

Ion source temperature: 200 ℃

Solvent delay: 6 min

Mass spectrum acquisition time: 6 min~12 min

Acrylamide monitoring ions: m/z 106, 133, 150 and 152

Quantitative ion: m/z 155

13.3 Formulation of standard curves

Infuse the derivative standard work liquid into Gas Chromatography-Mass Spectrometry System to detect the peak area of corresponding acrylamide and their internal standard. Linearity curves shall be mapped by taking the acrylamide sampling concentration of all standard work liquid(μg/L)as the abscissa and the peak area of 13C3 acrylamide internal standard detected on the quantitative ion quality chromatogram as the ordinate.

13.4 Determination of sample solution

Infuse the derivative sample solution into the Gas Chromatography-Mass Spectrometry System and measure the peak area ratio of acrylamide and 13C3 acrylamide internal standard. Acrylamide sampling concentration (μg/L) in the to-be-tested liquid is acquired according to standard curves. Parallel determination shall be done twice or more.

13.5 Mass spectrometric analysis

Infuse the samples and standard work liquid into the Gas Chromatography/Mass Spectrometer in sequence to record the total ion chromatogram and mass spectrum (see Fig A.3 to A.4 at Appendix A) , as well as the peak area of acrylamide and internal standard and keep the abundance qualification of time and fragment ions. The chromatographic peak SNR of detected acrylamide shall be more than 3. The retention time of target compounds in detected samples shall be in accordance with that in the standard solution. The abundance ratio of the relevant monitoring ions of the target compounds in tested samples shall be in accordance with that in the standard solution. See the permissible deviation at Table 1.

14 Expression of the analysis results

The acrylamide content in the samples shall be calculated according to the Formula (1) Internal Standard Method.

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Calculation results shall be shown with the arithmetic mean value of two individually analysis results acquired on a repetitive basis. The outcome shall be calculated to a three-effective-digit number (or to the first decimal place).

15 Precision

The absolute difference of the two independent analysis results acquired on a repetitive basis shall not exceed the 20% of the arithmetic mean value.

16 Others

Limit of quantitation is 10 μg/kg.

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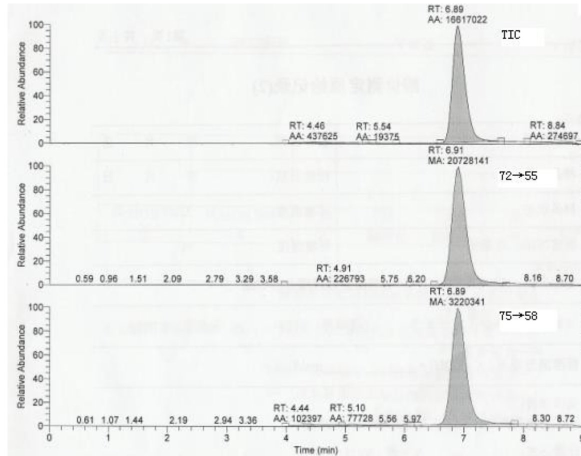
Appendix A:

Chromatogram and mass spectrum

A.1 Fig A.1 and A.2 are mass chromatogram and mass spectrum that are detected with LC-MS/MS.



Notes: From up to down is Total Ion Chromatogram (TIC), Acrylamide Selected Ion Flow Diagram(72→55)and 13C3-acrylamide Internal Standard Selected Ion Flow Diagram(75→58).

Fig A.1 Mass chromatogram of acrylamide and isotope internal standard 13C -acrylamide in potato chips

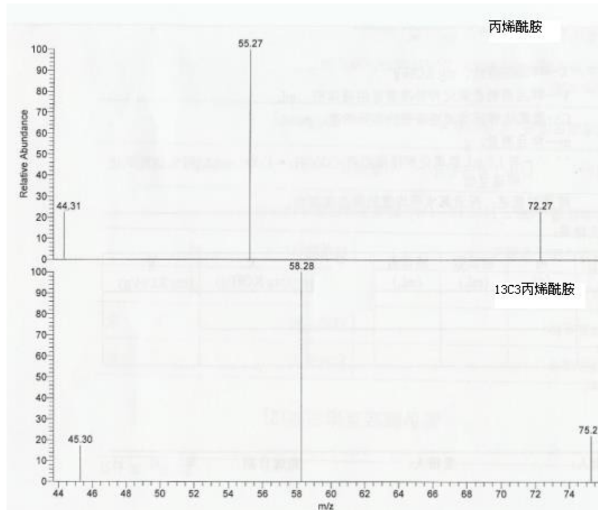


Fig A.2 Mass spectrum of acrylamide and internal standard 13C -acrylamide

A.2 Fig A.3 and A.4 are mass chromatogram and mass spectrum that are detected with LC-MS/MS.

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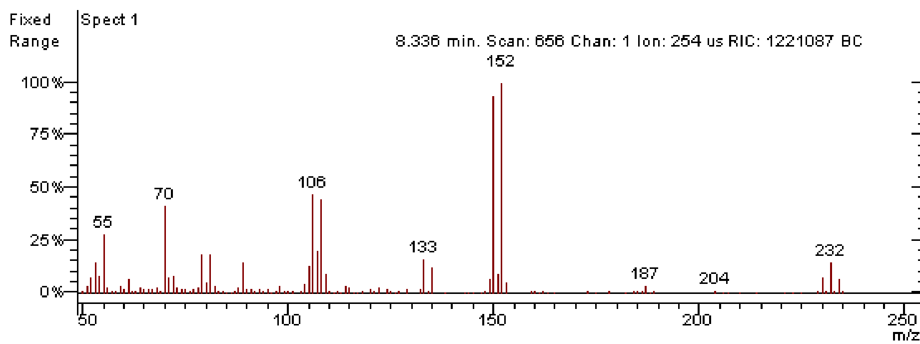
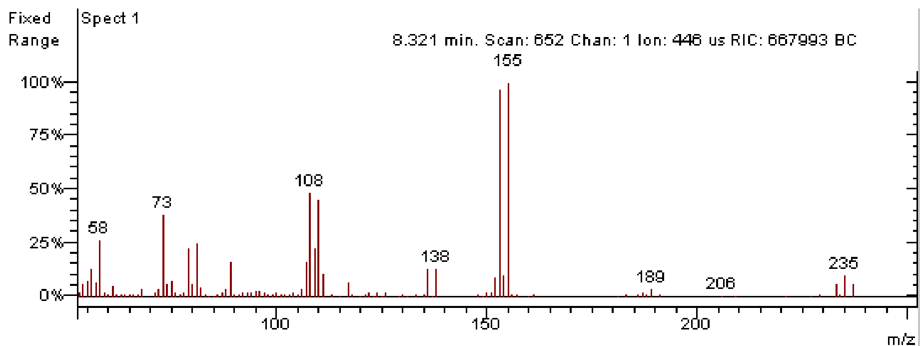
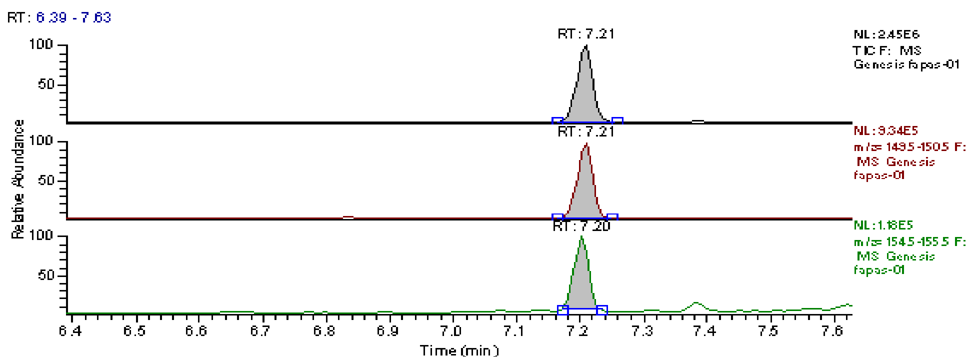




Notes: the above figure:acrylamide;the below figure:13C3-acrylamide

Fig A.3 GC-MS full scan mass spectrum of bromo-derivation in the standard solution



Notes: From up to down is Total Ion Chromatogram (TIC) and mass chromatogram of acrylamide derivative m/z 150 and 13C3-acrylamide derivative m/z 155.

Fig A.4 GC-MS mass chromatogram (Quadrupole) of potato chip samples

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