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**ARTICLE** *in* ANALYTICAL CHEMISTRY · SEPTEMBER 2013

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# Simultaneous Analysis of Acrylamide and Its Key Precursors, Intermediates, and Products in Model Systems by Liquid Chromatography–Triple Quadrupole Mass Spectrometry

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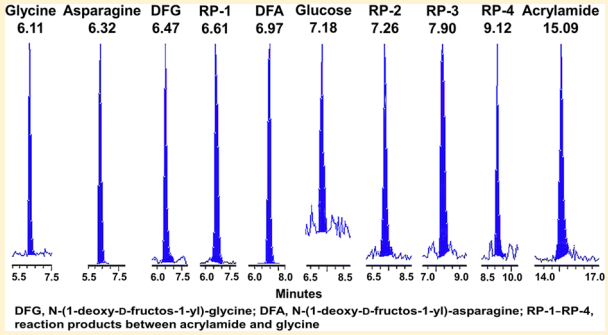
Supporting Information

**ABSTRACT:** A new analytical setup allowing the simultaneous analysis of precursors, intermediates and products in Maillard reaction model systems was developed to investigate the formation and mitigation kinetics of acrylamide. It was based on high-performance liquid chromatography combined with electrospray ionization triple quadrupole tandem mass spectrometry (HPLC–MS/MS). Chromatography and mass spectrometry conditions were optimized to permit simultaneous monitoring of compounds relevant to acrylamide, such as asparagine, glucose, glycine, *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG), *N*-(1-deoxy-D-fructos-1-yl)-asparagine (DFA), and four reaction products between acrylamide and glycine. A fairly good separation was achieved on a Venusil MP-$C_{18}$ column using an isocratic eluent of 0.5% formic acid in water at a flow rate of 0.4 mL/min. Triple quadrupole mass spectrometry in multiple reaction monitoring mode (MRM) was applied to obtain good sensitivity. All of the ten key reaction compounds were separated and determined in a single run within 15 min. High correlation coefficients ($r > 0.99$) of the ten analytes were obtained in their respective linear ranges. The method was capable to accurately quantify the ten key compounds in model systems during heating. The results showed that acrylamide formed in Maillard reaction could be reduced by reacting directly with glycine, and acrylamide concentration constantly decreased by about 60% within the 180 min heating at 150 °C when glycine was equal to asparagine and glucose in molecular concentration in model systems. This method helps achieve reduction in both time and labor of analysis of a large number of samples.




Acrylamide, a thermal process-induced contaminant in food, has attracted worldwide researchers to study the mechanism of its formation in foods, the risks associated for consumers and possible strategies to lower acrylamide levels in foodstuffs,[1] since acrylamide is a neurotoxic compound classified as a probable human carcinogen and genotoxicant.[2] It is well-known that Maillard reaction between asparagine and reducing sugar is the main pathway for the formation of acrylamide.[3,4] The yield of acrylamide was sensitive to the composition of free amino acid and sugar in food and to conditions which are beneficial to promote Maillard reaction, such as high temperature and low moisture level.[5] Therefore, the formation of acrylamide is one of a number of competing processes as the desired consequences of the Maillard reaction (color and flavor) share intermediates with acrylamide formation.

Taking the factors of effecting acrylamide formation into account, several effective measures have been suggested to reduce acrylamide levels during food processing, such as reducing the contents of precursors,[6] mitigating formation of acrylamide by adding other compounds,[7−10] and adjusting process parameters.[11] In particular, the acrylamide content could be reduced significantly when some amino acids other than asparagine were added.[12] It is more practical for the food industry to add amino acids to inhibit the formation of acrylamide among the strategies, since this operation would raise little concern on the artificial chemicals in foods.

Glycine is the smallest of the 20 amino acids commonly found in proteins and has been shown to be one of the most effective amino acid additives in mitigating acrylamide formation in bread, potato crisps, wheat flake, biscuit, and cracker at lab and pilot scale.[13−15] It has been proved that

**Received:** July 2, 2013
**Accepted:** September 4, 2013
**Published:** September 4, 2013

   dx.doi.org/10.1021/ac4019928 | *Anal. Chem.* 2013, 85, 9262–9271

competition of glycine and asparagine for the reducing sugars was responsible to decrease acrylamide in our previous study.[16] Additionally, our research team first proposed the Michael addition reactions between acrylamide and glycine with or without the initial oxidation of glycine were the direct pathways for the removal of acrylamide by glycine, and provided the direct evidence for the pathways by the unequivocal identification of the main reaction products between acrylamide and glycine and intermediates formed in acrylamide/glycine model systems.[17]

The knowledge of reaction kinetics is essential for understanding the acrylamide formation and elimination process. Thus it badly needs an accurate and rapid analytical method. The simultaneous quantification of multicomponents including precursors, intermediates, as well as products in complex reactions network is crucial to obtain concentrations of key compounds rapidly and precisely. Although multiresidues simultaneous analysis methods have been developed in food, environmental monitoring, and metabolite analysis,[18−21] methods for simultaneous determination of main reaction components in successive critical reaction stages of the complex chemical reactions are limited. Nikoline et al[22] developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to simultaneously determine acrylamide and its precursors (asparagine and reducing sugar). However, the accuracies and precisions in determinations of saccharides were expected to be improved. Zhang and co-workers[23] reported an efficient ultra high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method for the simultaneous analysis of acrylamide and its precursors in Maillard reactions using an isotope dilution technique. The method did not yet deal with the determination of intermediates and the products related to the elimination of acrylamide after its formation.

The aim of this study was to develop a high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method to simultaneously quantify ten key compounds in one run: precursors (asparagine, glycine, glucose), two intermediates *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG) and *N*-(1-deoxy-D-fructos-1-yl)-asparagine (DFA), acrylamide, and four reaction products (RP-1, RP-2, RP-3, and RP-4) in model systems for mitigating acrylamide by glycine. The developed method would have the potential to generate data rapidly, to provide a tool performing thorough research on the mitigating mechanism by glycine, and to monitor reaction process.

## EXPERIMENTAL SECTION

**Chemicals and Materials.** LC solvents were of HPLC grade and purchased from Honeywell (Seoul, South Korea). Formic acid (≥98%) was obtained from Sigma-Aldrich (Steinheim, Germany). Acrylamide (>99.9%) was purchased from Bio Basic Inc. (Markham, Canada), L-asparagine and glycine (≥99%) from Amresco (Solon, U.S.A.), and D-glucose from J&K Scientific Ltd. (Beijing, China). Ion-exchange resin (Dowex 50W × 8, $H^+$) was purchased from Huaerbo Ltd. (Beijing, China). $Na_2S_2O_5$-sodium disulphite, anhydrous alcohol, and aqueous ammonia (≥25−28%) were of analytical grade and obtained from Beijing Chemicals Co. (Beijing, China).

**Preparation of Intermediates.** DFG and DFA were prepared according to the method described by Martins et al[24] with the modification that mass spectrometer was used to check the presence of DFG and DFA. For DFA, L-asparagine (10.59 g, 0.07 mol), D-glucose (21.02 g, 0.28 mol), and $Na_2S_2O_5$-sodium disulphite (7.6 g, 0.04 mol) were added to 80 mL of MeOH−water solution (1:1). The mixture was then stirred for 20 min at room temperature and subsequently refluxed for 14 h. Purification methods was same to that of DFG.[24] Analog calculation for $C_{10}H_{18}N_2O_8$ (DFA): C, 40.82; H, 6.17; N, 9.52. Found: C, 38.70; H, 6.59; N, 8.12.

**Preparation of Standard Solutions.** Standard substances of acrylamide, asparagine, glycine, and glucose were commercial products and that of reactions products were prepared on the basis of our previous work.[17] Stock solutions and calibration standards of all ten analytes were prepared in water.

**HPLC-MS/MS Analysis.** The ten analytes were determined by an Alliance 2695 Separations Module (Waters, Milford, MA, U.S.A.) coupled to a Micromass Quattro Micro triple-quadrupole mass spectrometer (Micromass Company Inc., Manchester, U.K.). HPLC separation was carried out on a Venusil MP-$C_{18}$ column (250 mm ×4.6 mm i.d., 5 μm particle size, Agela Technologies, Newark, USA) maintained at 30 °C. The mobile phase was 0.5% formic acid in water at a flow rate of 0.4 mL/min. Detection was performed by MS/MS after selection and optimization of multiple reaction-monitoring mode (MRM) traces from MS and daughter scans. The optimized MS instrument parameters obtained after tuning were as follows: capillary voltage, 3 kV; source temperature, 110 °C; desolvation temperature, 400 °C; desolvation gas flow, 600 L/h nitrogen; and cone gas flow, 50 L/h; argon collision gas pressure to $2 \times 10^{-3}$ mbar for MS/MS. In the MRM transitions, the dwell and inter scan time were 0.4 and 0.1 s, respectively. Data acquisition was performed with MassLynx software (version 4.1, Micromass, Manchester, U.K.). The cone voltage and collision energy for each monitored transition of the ten analytes were shown in Table 1. The collision energy of the analytes was optimized according to the maximal abundance of their quantitative ions.

**Table 1. Parameters of MRM Mode to Acquire Data**

| | traces ($m/z$ units) | cone voltage (V) | collision energy (eV) |
|---|---|---|---|
| AA $[M + H]^+$ | 72 > 55 | 20 | 13 |
| Gly $[M + H]^+$ | 76 > 30 | 20 | 35 |
| Asn $[M + H]^+$ | 133 > 74 | 15 | 15 |
| Glc $[M + Na]^+$ | 203 > 143 | 32 | 20 |
| DFG $[M + H]^+$ | 238 > 220 | 12 | 10 |
| DFA $[M + H]^+$ | 295 > 259 | 13 | 15 |
| RP-1 $[M + H]^+$ | 147 > 88 | 18 | 15 |
| RP-2 $[M + H]^+$ | 218 > 159 | 22 | 15 |
| RP-3 $[M + H]^+$ | 205 > 88 | 30 | 10 |
| RP-4 $[M + H]^+$ | 276 > 217 | 30 | 10 |

**Analysis of Samples.** An aliquot of reaction solution (1.0 mL) from acrylamide/glycine (AA/Gly), asparagine/glucose (Asn/Glc), glycine/glucose (Gly/Glc), and asparagine/glucose/glycine (Asn/Glc/Gly) model systems heating at 150 °C from 15 to 180 min were passed through 0.22 μm filtration membranes, and diluted with the mobile phase. The filtrate was transferred to an autosampler vial for HPLC-MS/MS analysis.

## RESULTS AND DISCUSSION

**Optimization of LC Conditions.** The simultaneous analysis performed on the present chromatographic system


dx.doi.org/10.1021/ac4019928 | Anal. Chem. 2013, 85, 9262−9271

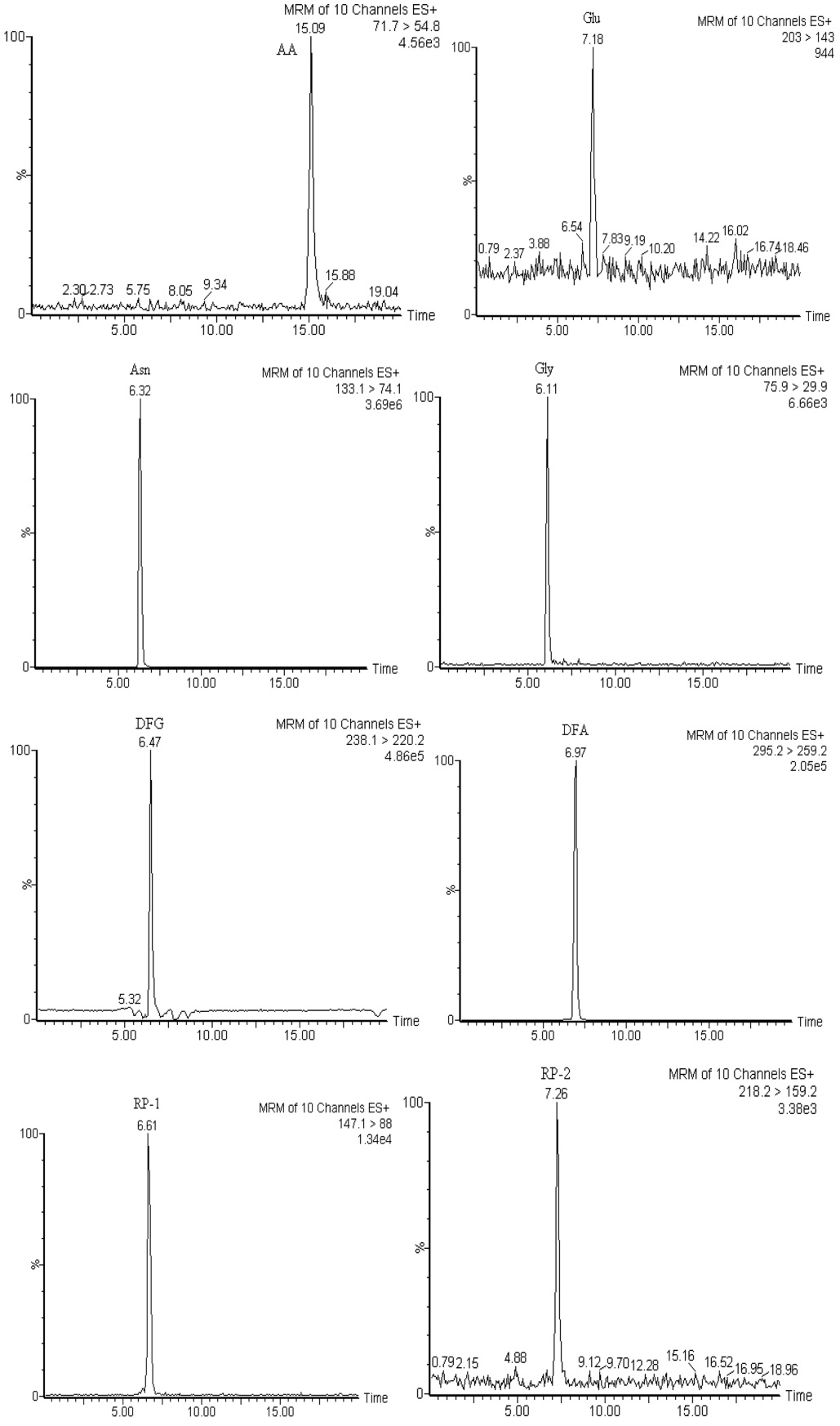



**Figure 1.** continued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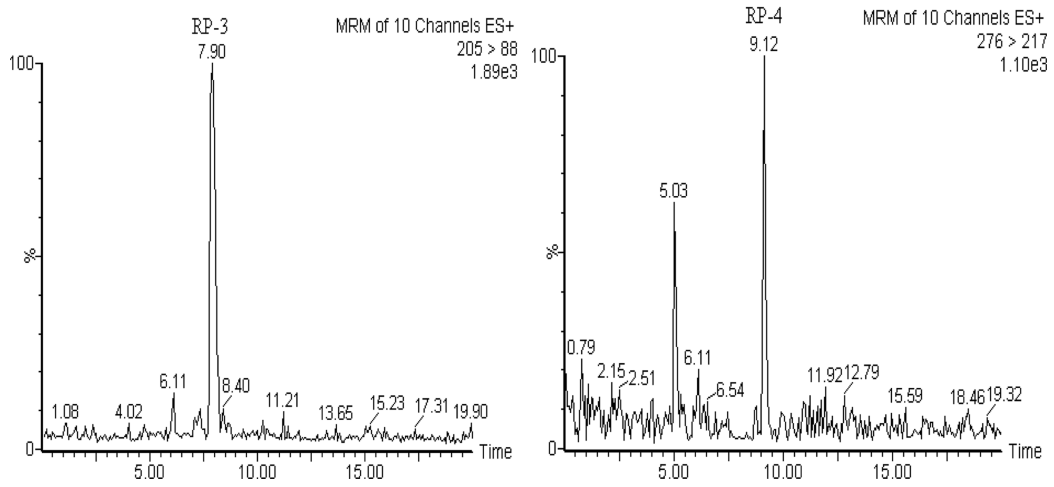


**Figure 1.** MRM chromatograms of simultaneous determination of the ten analytes. AA, acrylamide; Glu, glucose; Asn, asparagine; Gly, glycine; DFG, *N*-(1-deoxy-D-fructos-1-yl)-glycine; DFA, *N*-(1-deoxy-D-fructos-1-yl)-asparagine; RP-1−RP-4, four reaction products between acrylamide and glycine. The concentrations of analytes were at levels when Asn/Glu/Gly model systems heated for 15 min: $C_{AA}$ = 0.05 mM, $C_{Glu}$ = 61.55 mM, $C_{Asn}$ = 100.01 mM, $C_{Gly}$ = 100.21 mM, $C_{DFG}$ = 0.24 mM, $C_{DFA}$ = 0.043 mM, $C_{RP\text{-}1}$ = 0.023 mM, $C_{RP\text{-}2}$ = 0.0049 mM, $C_{RP\text{-}3}$ = 0.091 mM, $C_{RP\text{-}4}$ = 0.14 mM.

seems difficult because of the significant difference in polarity among the ten compounds. Besides, the separation among the components with the adjacent retention time is a great challenge. Therefore, the selection of the chromatographic column and mobile phase was a prerequisite to obtain a good separation. Acrylamide is a highly polar molecule with poor retention in conventional LC reversed phase sorbents. Therefore, based on our experience on chromatographic separation of acrylamide[25] and the relationships among structures of the ten compounds, several columns specially designed to have enhanced retention and selectivity for polar compounds were tested for their separation efficiencies: (i) Thermo Hypersil ODS-$C_{18}$ column (250 mm ×4.6 mm i.d., 5 $\mu$m), (ii) Waters Atlantis d$C_{18}$ column (150 mm ×2.1 mm i.d., 3 $\mu$m), (iii) Agela Venusil MP-$C_{18}$ column (250 mm ×4.6 mm i.d., 5 $\mu$m), and (iv) Agela HILIC column (250 mm ×4.6 mm i.d., 5 $\mu$m). The peak elution time of acrylamide on Atlantis d$C_{18}$ column was about 3 min, which was faster than that on other three columns. While the amino acids and reactions products were difficult to be retained and separated efficiently on Atlantis d$C_{18}$ column as its column length was limited. RP-1 and RP-2 were not separated completely on HILIC column and ODS-$C_{18}$ column. So Venusil MP-$C_{18}$ was selected as the separation column with a flow rate of 0.4 mL/min which matched with the MS/MS electroscopy ionization.

Besides the optimization of separation efficiencies of the chromatographic column, the choice of mobile phase should be concerned based on the ionization efficiency of analytes in MS/MS system in order to obtain nice resolution and high sensitivity. The mobile phase was adjusted from the mixture of 10% acetonitrile and 90% water (used to analyze acrylamide in our early study)[25] to 100% aqueous mobile phase. Results showed that the retention of acrylamide could be improved and the MS sensitivity did not change significantly by avoiding organic modifiers like acetonitrile and methanol in the aqueous mobile phase. Venusil MP-$C_{18}$ can be used with pure water as the mobile phase without suffering from the "phase collapse" phenomena associated with traditional reversed phase columns. Therefore, the combination of Venusil MP-$C_{18}$ column and water as a mobile phase gave good retention of polar compounds and maintain good peak shapes and intensities. Mobile phase containing organic acid can often improve ionization when using electrospray, and consequently increase the sensitivity of a method. Results from the MS full scan of ten compounds showed that the responses of corresponding molecular ions under the $ESI^+$ mode were greatly improved and stabilized, and high sensitivity was subsequently obtained when formic acid was added. Finally, water containing 0.5% formic acid as the mobile phase appeared optimal with regard to retention, separation efficiency, and MS response of compounds.

The chromatograms of ten analytes obtained from Asn/Glc/Gly model systems heated 15 min were shown in Figure 1. Glycine, asparagine, DFG, RP-1, DFA, glucose, RP-2, RP-3, RP-4, and acrylamide successively eluted from the column. The whole separation of the all ten analytes was completed when acrylamide eluted at about 15 min in a chromatographic cycle. Though the retention time of glucose, asparagine, glycine, DFG, DFA, RP-1, and RP-2 distributed around 6−7 min closely, the characteristic ions of each compound were different and there was no interference among each other for the quantification of the seven compounds in MRM mode.

**Optimization of MS/MS Conditions.** In this study, triple quadrupole mass spectrometry was applied to increase sensitivity in the determination. As the presence of nitrogen atom(s), having an important proton affinity (basic character), and carboxyl groups (acidic character) in the ten compounds except glucose, the MS spectra could be acquired in both positive and negative ion modes. While most of them could be monitored under both $ESI^+$ and $ESI^-$ modes, the stronger response was observed under the $ESI^+$ mode than $ESI^-$ mode, such as asparagine described by others.[22,23] The fragmentations of the corresponding characteristic ions used to quantification in MRM were presented in Figure 2.

The ion fragments of acrylamide were in agreement with the previous work.[26] The fragment ion $m/z$ 55 showed a relatively high intensity and was selected to quantify acrylamide. Saccharides could be ionized as sodium ion adducts without the addition of sodium ions into the mobile phase in advance and the sodium ion adducts are usually more abundant than the hydrogen ion adducts. Hence the glucose was detected as sodium adducts. Ions $[M + Na]^+$ at $m/z$ 203 indicated the


dx.doi.org/10.1021/ac4019928 | *Anal. Chem.* 2013, 85, 9262−9271

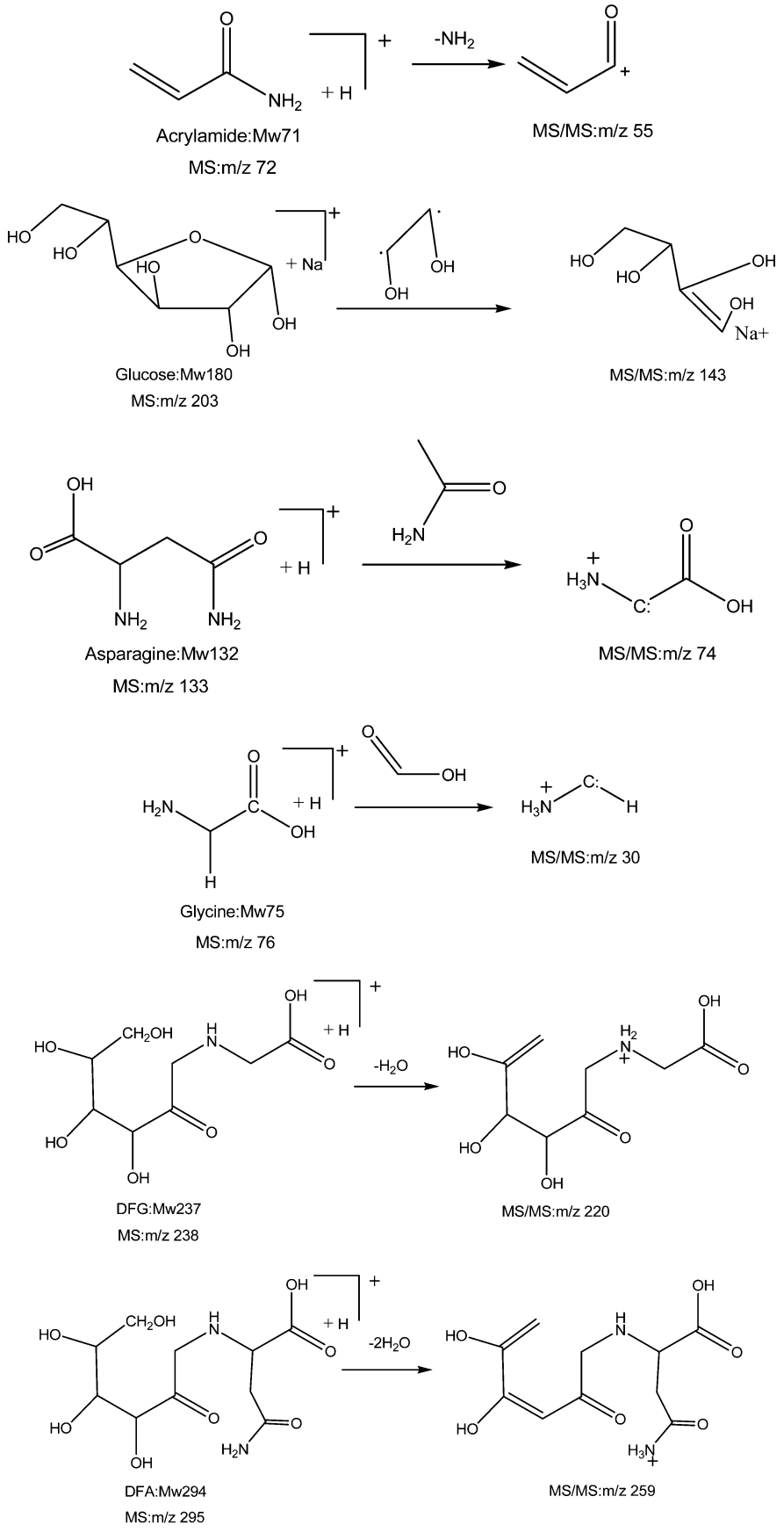


**Figure 2.** continued


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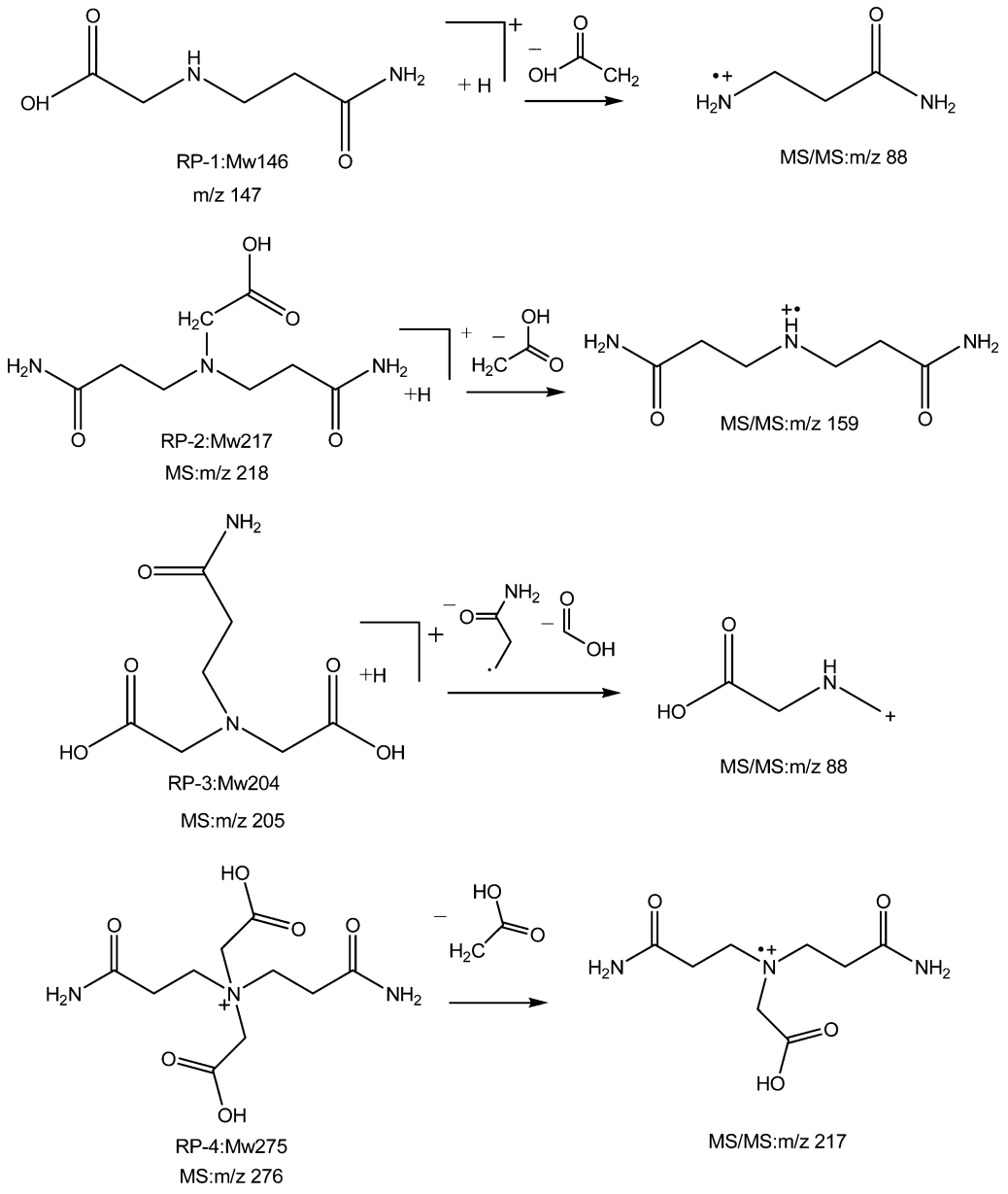


**Figure 2.** Fragmentation of the ten analytes. DFG, *N*-(1-deoxy-D-fructos-1-yl)-glycine; DFA, *N*-(1-deoxy-D-fructos-1-yl)-asparagine; RP-1−RP-4, four reaction products between acrylamide and glycine.

formation of sodium ion adducts of glucose, which was also detected by Nielsen[22] in a LC-MS/MS method for simultaneous analysis of acrylamide and its precursors. The inferior signal-to-noise ratio in the MRM chromatography of glucose at significant levels showed that the monosodiated adduct ions $[M + Na]^+$ was unstable, although their response was high in full scan. The fragment ion $m/z$ 74 of asparagine corresponded to the loss of acid amide ($-CH_2CONH_2$). The similar fragmentation of glycine was observed that ion $m/z$ 30 corresponded to the loss of carboxyl group (−COOH) in the present work. The quantitative ions of both DFG and DFA corresponded to the loss of water from the molecular ions, respectively. The characteristic ions of the reactions products have been discussed in our previous work[17] and their quantitative ions were listed in Table 1.

**Calibration and Validation.** The quantification of all compounds was achieved by means of seven-point calibration curves. All calibration curves were not forced through the origin and the regression lines were calculated by no weight. The concentrations of the standards were selected in a way that they could cover a wide range of concentrations detected. Calibration standards solutions were 1−15 μmol/L for acrylamide, 10−40 μmol/L for asparagine, 100−500 μmol/L for glycine, 50−550 μmol/L for glucose, 1−15 μmol/L for DFG, 1−15 μmol/L for DFA, 1−15 μmol/L for RP-1, 0.05−0.25 μmol/L for RP-2, 2−25 μmol/L for RP-3, and 2−20 μmol/L for RP-4. Strong linear relationships and good coefficients of determination were achieved for all ten compounds over the concentration range (Table 2).

Samples were spiked with the standards of ten analytes at different low, intermediate and high levels to determine their recoveries, respectively. The low, intermediate, and high spiking levels of acrylamide, asparagine, DFG, DFA, RP-1, RP-3, and RP-4 standards were 2, 10, and 20 μmol/L, respectively. Meanwhile, the low, intermediate, and high spiking levels of glycine and glucose were 50, 200, and 500 μmol/L, and those


dx.doi.org/10.1021/ac4019928 | Anal. Chem. 2013, 85, 9262−9271

**Table 2. Calibration Results for Ten Analytes**[a]

| analyte | linear range (μmol/L) | slope | y-intercept | coefficient($r$) |
|---|---|---|---|---|
| acrylamide | 1−15 | 1774.13 | 218.18 | 0.99907 |
| asparagine | 10−40 | 1098.41 | 3109.24 | 0.99861 |
| glycine | 100−500 | 1.27 | 51.71 | 0.99099 |
| glucose | 50−550 | 0.29 | 24.93 | 0.99758 |
| DFG | 1−15 | 5688.61 | 4876.55 | 0.99958 |
| DFA | 1−15 | 3649.93 | 792.32 | 0.99870 |
| RP-1 | 1−15 | 4754.44 | 2672.74 | 0.99804 |
| RP-2 | 0.05−0.25 | 16409.51 | −21.02 | 0.98857 |
| RP-3 | 2−25 | 655.67 | −745.89 | 0.99415 |
| RP-4 | 2−20 | 84.56 | −72.55 | 0.99326 |

[a]DFG, *N*-(1-deoxy-D-fructos-1-yl)-glycine; DFA, *N*-(1-deoxy-D-fructos-1-yl)-asparagine; RP-1−RP-4, four reaction products between acrylamide and glycine.

levels of RP-2 were 0.05, 0.1, and 0.2 μmol/L. The recoveries of all analytes ranged from 96.9% to 108.2% at three spiking levels (Supporting Information Table S1).

LOD and LOQ were determined as the amounts of analytes that produced a signal-to-noise ratio of 3:1 and 10:1, respectively. The values of LOD of acrylamide, asparagine, DFG, DFA, RP-1, RP-2, and RP-3 were less than 10 ng/mL, and that of glycine and glucose were at levels of mg/mL (Supporting Information Table S1).

Precision was calculated in terms of intraday repeatability and interday reproducibility as RSD% at three concentration levels. The reaction solutions were sampled in 15, 30, and 45 min heating for the detection of DFG and DFA, and in 60, 120, and 180 min heating for the other eight compounds, respectively. The values of RSD ranged from 0.0% to 10.3% for the intraday precision test ($n$ = 6) and 0.1% to 10.4% for the interday precision tests ($n$ = 6) (Supporting Information Table S2).

**Analysis of Ten Analytes and Kinetic Evaluation.** As the main pathway for the formation of acrylamide in foods is related to the Maillard reation between asparagine and reducing sugar, the effect of glycine on the formation and reduction of acrylamide should be investigated in Asn/Glc/Gly model systems. At the same time, the changes in concentrations of compounds related to acrylamide should be compared in AA/Gly, Asn/Glc, and Gly/Glc model systems.

Results appearing from Figure 3−5 showed the variance of precursors, intermediates, and products in different model systems with heating time. The kinetics of the ten compounds was clearly profiled. The changes of acrylamide, glycine, and reactions products in AA/Gly model systems have been discussed in previous work.[17] In the absence of heating (heating time, 0 min), the concentrations of three precursors (asparagine, glucose, and glycine) were quantified as approximately 0.1 mol/L, which is the addition level used in this study, while acrylamide could not be detected, indicating there was no contamination in the experiments.

The concentrations of acrylamide in both Asn/Glc/Gly and Asn/Glc model systems were increased with heating time, and that in Asn/Glc/Gly model systems were always about half of that in Asn/Glc model systems (Figure 3A). If the concentrations of acrylamide in Asn/Glc/Gly and Asn/Glc model systems were set as $C_{AA3}$ and $C_{AA2}$ respectively, the inhibitory rate ($\gamma_I$) of acrylamide by glycine was $\gamma_I = [(C_{AA2} - C_{AA3})/C_{AA2}] \times 100\%$. The inhibitory rate varied between 55% and 64% within the 180 min reaction time (Figure 3B). It suggested that the obvious mitigating effect of glycine on acrylamide in complex reactions was constant even with the dynamic glycine concentrations.

The concentrations of asparagine decreased more than 90% up to 135 min and then changed little in both model systems (Figure 4A). Especially in the first 60 min, the first order observed rate coefficient ($k$) for asparagine loss in Asn/Glc/Gly model systems ($k$ = 0.75) was significantly less than that in Asn/Glc model systems ($k$ = 1.91), indicating the competition of glycine with asparagine for the reducing sugars occurred in Asn/Glc/Gly model systems.

Glycine decreased by about 10% up to 180 min in both Asn/Glc/Gly and Gly/Glc model systems (Figure 4B). In Asn/Glc/Gly model systems, the glycine was involved in competing with asparagine for glucose and combining the formed acrylamide. So the $k$ of glycine in Asn/Glc/Gly model systems ($k$ = 0.07)

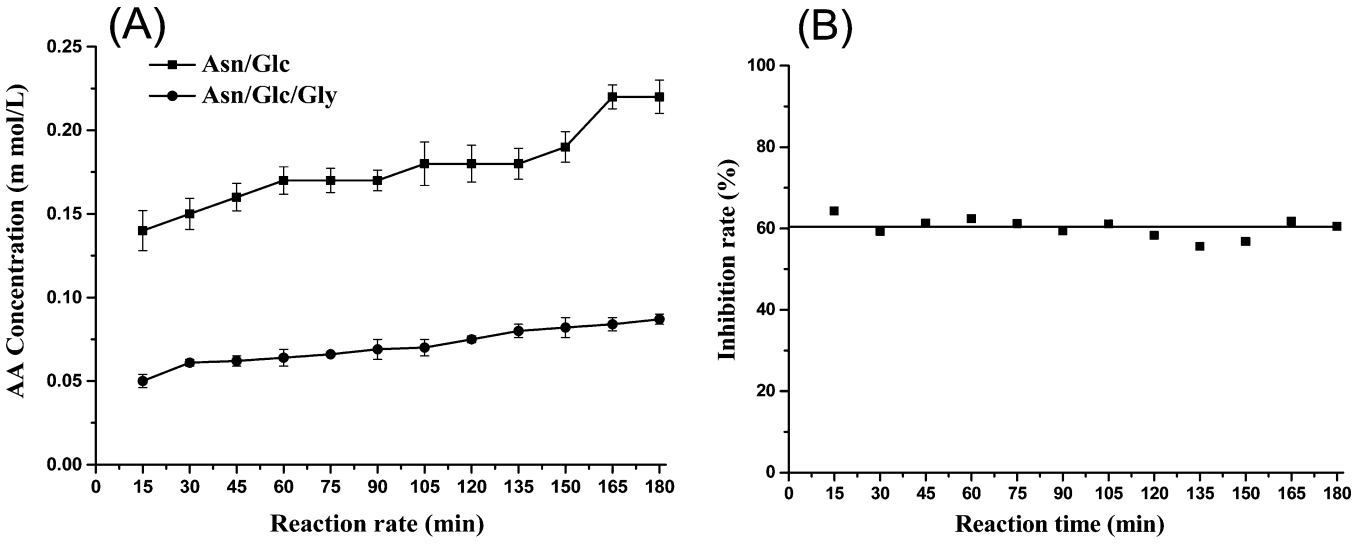


**Figure 3.** Time courses of AA in Asn/Glc and Asn/Glc/Gly model systems (A) heated at 150 °C with different heating time. Each experiment was performed in triplicate repeats ($n$ = 3). Inhibition rates of AA (B) by adding Gly in Asn/Glc/Gly model systems were obtained in comparison with that in Asn/Glc model systems. AA, acrylamide; Asn, asparagine; Glc, glucose; Gly, glycine.


dx.doi.org/10.1021/ac4019928 | *Anal. Chem.* 2013, 85, 9262−9271

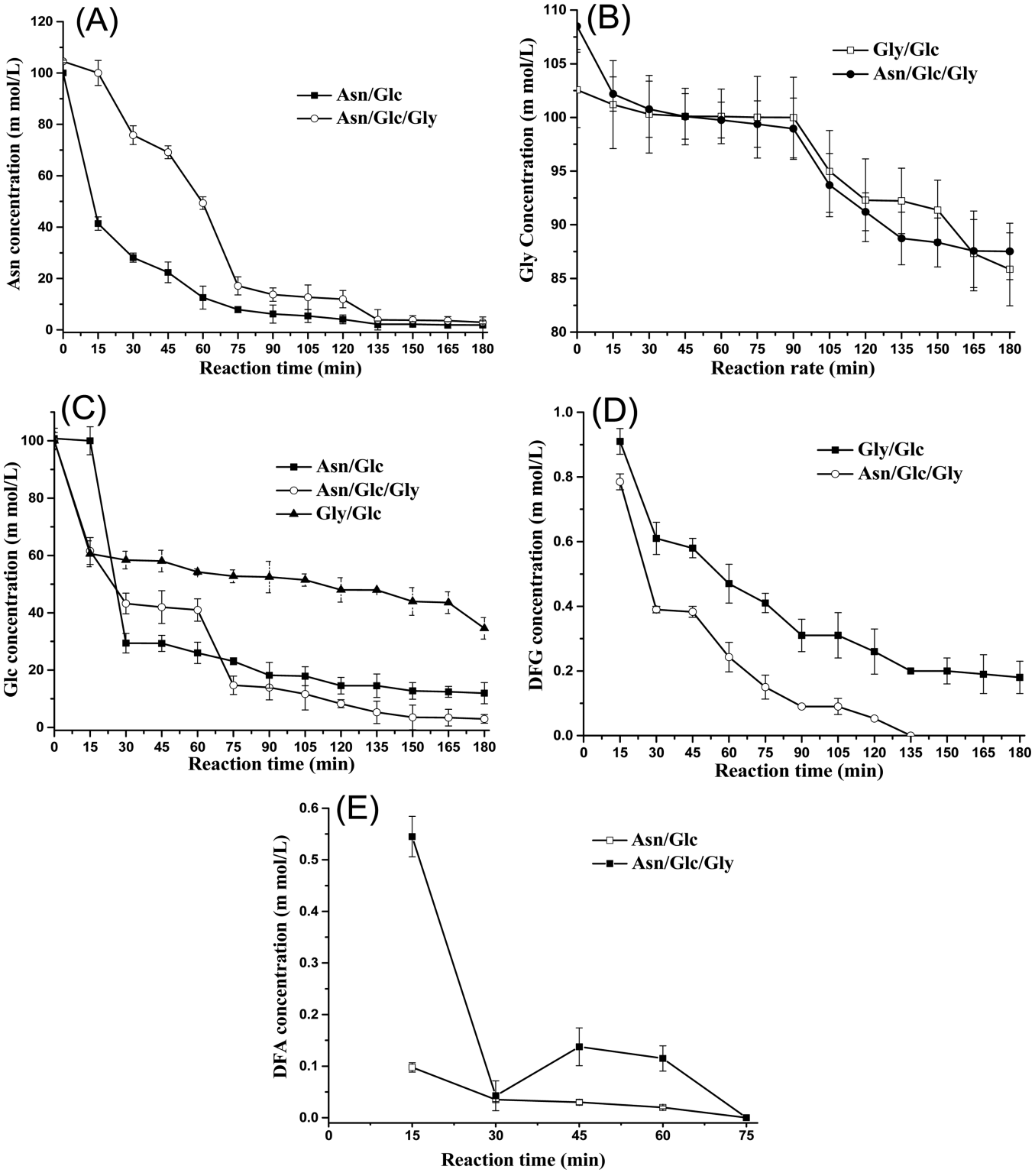


**Figure 4.** Time courses of Asn (A), Gly (B), Glc (C), DFG (D), and DFA (E) in Asn/Glc, Gly/Glc, and Asn/Glc/Gly model systems heated at 150 °C with different heating time. Each experiment was performed in triplicate repeats ($n$ = 3). Asn, asparagine; Gly, glycine; Glc, glucose; DFG, *N*-(1-deoxy-D-fructos-1-yl)-glycine; DFA, *N*-(1-deoxy-D-fructos-1-yl)-asparagine.

was slightly greater than that ($k$ = 0.06) in Gly/Glc model systems.

The concentrations of glucose decreased more than 90% in Asn/Glc/Gly model systems, 80% in Asn/Glc model systems, and 50% in Glc/Gly model systems up to 180 min, respectively (Figure 4C). After the reaction lasting for 70 min, the concentrations of glucose in Asn/Glc/Gly model systems were less than that in Asn/Glc and Glc/Gly model systems. It was the result of glycine and asparagine consuming glucose together in Asn/Glc/Gly model systems.

In Asn/Glc/Gly model systems, the concentrations of asparagine and glucose were quickly reduced in the first 75


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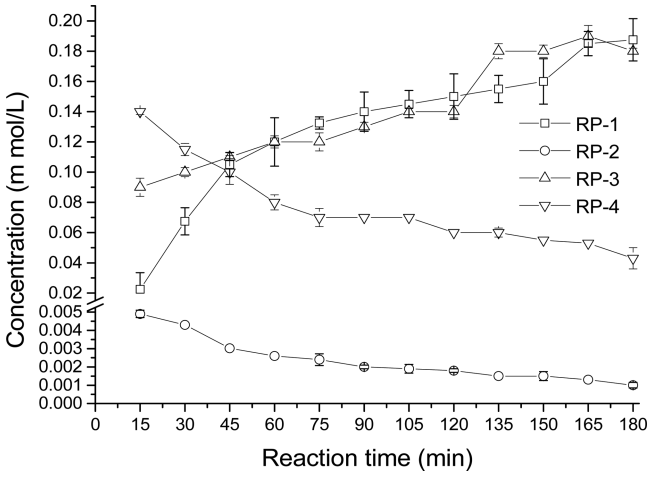


**Figure 5.** Time courses of RP-1, RP-2, RP-3, and RP-4 in Asn/Glc/Gly model systems heated at 150 °C with different heating time. Each experiment was performed in triplicate repeats ($n$ = 3). RP-1−RP-4, four reaction products between acrylamide and glycine.

min heating, and then decreased gradually slowly with the development of reaction. The $k$ of glucose ($k$ = 0.87) was faster than that of asparagine ($k$ = 0.75), because glucose was simultaneously involved in the isomerization and Maillard reactions with glycine and asparagine. The loss of glycine was far less than that of asparagine in the whole process, and $k$ of glycine in Gly/Glc model systems ($k$ = 0.06) was significantly less than that of asparagine in Asn/Glc model systems ($k$ = 1.91), indicating that asparagine had the advantage in competition with glycine for glucose.

The concentrations of the both intermediates (DFG and DFA) detected decreased with heating time after their formation indicating that they generated fast and were unstable. DFG in Asn/Glc/Gly model systems was completely consumed, and the reduction of that in Glc/Gly model systems became moderate up to 135 min (Figure 4D). The concentrations of DFG in Asn/Glc/Gly model systems were less than that in Glc/Gly model systems from beginning to end. In the meantime (0−135 min), the concentrations of glycine in both model systems were about the same amount (Figure 4B), but the glucose concentrations in Glc/Gly model systems was 40% higher than that in Asn/Glc/Gly model systems (Figure 4C). So the difference in the concentrations of DFG in both model systems was the result of the difference of the concentrations of glucose, indicating that asparagine competing with glycine for glucose and inhibiting the formation of DFG. DFA was completely consumed up to about 76 min in both Asn/Glc/Gly and Asn/Glc model systems (Figure 4E). The concentration of DFA at 15 min in Asn/Glc/Gly model systems was almost five times as much as that in Asn/Glc model systems, also indicating that glycine competing with asparagine for glucose and inhibiting the formation of DFA. The ending time suggested that DFG was more stable than DFA.

The concentrations of RP-1 and RP-3 increased with heating time, whereas the concentrations of RP-2 and RP-4 decreased with heating after their formation in Asn/Glc/Gly model systems (Figure 5). The concentrations of RP-2 were the least compared with that of the other three reactions products. The concentrations of the four reactions products increased linearly with heating time in AA/Gly model systems.[17] The different kinetic profiles of RP-1 and RP-2 in Asn/Glc/Gly model systems showed that the reaction between RP-1 and acrylamide might be disturbed and the acrylamide concentration was so little that the production of RP-2 was limited. According to the structure of RP-2 and nucleophilic adduction, the ratio to produce RP-2 for glycine and acrylamide was 1: 2, indicating that excess acrylamide favors the formation of RP-2. While in the Asn/Glc/Gly model systems, glycine outweighed acrylamide greatly. So the concentration ratio of reactants made against the formation of RP-2 and the concentrations of RP-2 increased steeply within the initial 15 min followed by decrease with the development of reaction. It was same to the formation of RP-4 on the base of RP-3. Sufficient glycine favored the formation of RP-3 so the concentrations of RP-1 and RP-3 were about the same amount all the time. The difference in the changes of the four reaction products mean that acrylamide was not only formed from asparagine and glucose through Maillard reaction, but also might be released from RP-2 and RP-4 through Hoffmann elimination. Then acrylamide was used to form RP-1 and RP-3.

Compared to individual analyzing some of these analytes in previous work,[27] the current method greatly reduced the time and cost. Besides, the present method also eliminated the error of results from different quantitative methods. Although the matrix effect is one of the limitations of LC-MS/MS with electrospray interface (ESI) that accuracy, precision and robustness cannot be guaranteed, high multiples of dilution for samples eliminated matrix effect easily in this study.

## CONCLUSIONS

In conclusion, a sensitive, specific and accurate analytical method based on HPLC-MS/MS was developed and demonstrated in this work to be an excellent analytical tool to simultaneously monitor the key precursors, intermediates and products related to acrylamide in a single analytical run. Meanwhile, the quantitative results in model systems demonstrated that simple and rapid sample preparation in combination with high sensitivity of detection were the principal advantages of this method as compared to HPLC or GC. It could be applied to the trace analysis of the multiple key compounds in complex reactions for a large number of samples, generate kinetic data, and estimate reaction efficiency.

## ASSOCIATED CONTENT

### Supporting Information

Additional material as described in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

This study was financially supported by the National Natural Science Foundation of China (No.31222044 and No.31071554), the National Basic Research Program of China (973 Program, No. 2012CB720805), and the National High Technology Research and Development Program of China (863 Program, No. 2011AA100806).

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