**Determination of *N*7-glycidamide guanine adducts in human blood DNA following exposure to dietary acrylamide using LC-MS/MS.**

**Donald JL Jones§1,2, Raj Singh§1,2, Victoria Emms1, Peter B Farmer1,Derryn Grant1, Paulene Quinn2,3, Colleen Maxwell2,3, Antria Mina1, Leong L Ng2,3,Sandra Schumacher1 and Robert G Britton\*1**

**§ Joint First Authors**

**\* Corresponding Author**

**Address:**

1Leicester Cancer Research Centre, Leicester Royal Infirmary, University of Leicester, Leicester, LE1 5WW, United Kingdom

2Leicester van Geest Multi-Omics Facility, Hodgkin Building, University of Leicester, Leicester, LE2 7HR, United Kingdom

3Department of Cardiovascular Sciences, University of Leicester and National Institute for Health Research Leicester Biomedical Research Centre, Glenfield Hospital, Leicester, LE1 7RH, United Kingdom

**Abstract**

**Rationale**

Acrylamide is classified as a probable human carcinogen that is metabolised to glycidamide, which can covalently bind to DNA. The aim of this study was to investigate the formation of *N*7-glycidamide-guanine (*N*7-GA-Gua) adducts in human blood DNA following exposure to acrylamide present in carbohydrate rich foods as part of the normal human diet.

**Methods**

Lymphocyte DNA was extracted from blood samples obtained from healthy human volunteers. Following thermal depurination of the DNA samples, *N*7-GA-Gua adducts were quantified using a validated LC-MS/MS method incorporating a stable isotope-labelled internal standard. Estimated dietary acrylamide intake was recorded by completion of food frequency questionnaires for the 24 hours prior to volunteer blood donation.

**Results**

A LC-MS/MS method was validated with a limit of detection of 0.25 fmol and a lower limit of quantitation of 0.50 fmol on column.  *N*7-GA-Gua adducts were detected in human blood DNA with the levels ranging between 0.3 to 6.3 adducts per 108 nucleotides. The acrylamide intake was calculated from the food frequency questionnaires ranging between 20.0 and 78.6 µg.

**Conclusions**

Identification and quantification of *N*7-GA-Gua adducts in the blood DNA of healthy volunteers suggests that dietary acrylamide exposure may lead to the formation of DNA adducts.  This important finding warrants further investigation to ascertain a correlation between environmental/dietary acrylamide exposure and levels of DNA adducts.

**Introduction:**

Research initiated after the leak of a grouting agent into the local water table in southwest Sweden during tunnel building in 1997 identified acrylamide in some common foodstuffs 1. Acrylamide (AA) has been classified as a probable human carcinogen by the International Agency for Research on Cancer (IARC) and found to be present in relatively high concentrations in food (up to 1000µg AA per Kg of potato chips) 2. It is also a ubiquitous compound present in adhesives and grouts, in waste water treatments as well as found in the mining, oil, paper and cosmetic industries and is a component of tobacco smoke 3. Acrylamide is formed when foods with high starch content are cooked at elevated temperatures (T>120°C), for example, grilled, fried or baked. Foods that are particularly high in acrylamide include potato chips and crisps, bread, coffee and cereals. Acrylamide is formed in foods via the Maillard reaction between reducing sugars, such as glucose and the amino acid asparagine (Figure 1), which is abundant in carbohydrate-rich foods like potatoes 4,5. The presence of acrylamide in a variety of different food types has been confirmed using GC-MS and LC-MS/MS 6-9 with potato products, such as crisps and chips having the highest levels and significant amounts measured in bread, cakes, biscuits and coffee.

To date, there is no compelling evidence correlating dietary AA exposure to increased cancer incidence in human populations. In a 2015 systematic review and meta-analysis of epidemiological studies 10, it concluded that dietary acrylamide was not linked to an increased risk of most cancers, but “a modest association for kidney cancer, and for endometrial and ovarian cancers in never smokers only, cannot be excluded.” Data emerging from the Japan Public Health Centre-Based Prospective Study (JPHC) 11, which has collected information on lifestyle habits and health conditions since 1990 also shows no association between dietary acrylamide and increased cancer incidence in a number of cancers including prostate, bladder 12, colorectal 13, endometrial, and ovarian 14 cancer. Despite the lack of evidence to directly link dietary acrylamide to increased cancer risk in human studies, there is still a reluctance to declare acrylamide as ‘safe’, with the European Food Safety (EFSA) panel on contaminants in the food chain (CONTAM) 15 concluding, “although the epidemiological associations have not demonstrated AA to be a human carcinogen, the margins of exposure (MOEs) indicate a concern for neoplastic effects based on animal evidence.”

Acrylamide that is ingested can be rapidly absorbed through the intestinal tract and is metabolized via cytochrome P450 2E1 (CYP2E1) to the active metabolite, glycidamide (GA). Detoxifications of GA can occur via conjugation with glutathione 16. GA is a reactive epoxide which forms protein adducts 17 and DNA adducts 18,19. The two main DNA adducts detected in mice have been identified as *N*7-(2-carbomyl-2-hydroxyethyl) guanine (*N*7-GA-Gua) 18,19 (Figure 1) and *N*3-(2-carbomyl-2-hydroxyethyl) adenine (*N*3-GA-Ade) 18. A third adduct, the *N*1-(2-carbomyl-2-hydroxyethyl) adenine (*N*1-GA-Ade) is presumed to be generated as a consequence of dimroth rearrangement 18,20.

The formation of DNA adducts by genotoxic carcinogens is considered an essential event in the process by which such compounds may cause cancer. The determination of DNA adduct levels can potentially allow for carcinogenic risk assessment as well as determining exposure to genotoxic chemicals for an individual person. The use of mass spectrometry to analyse DNA adducts has been well established due to its sensitivity, specificity and accurate quantitation when incorporating stable isotope internal standards 21-23. LC-MS/MS methods have been developed and applied to the detection of acrylamide DNA adducts in animal tissue 18. *N*7-GA-Gua adducts have recently been quantified in the urine of workers exposed to AA 24,25, and in the blood of human volunteers 26 concurrently with this work. DNA adducts are formed endogenously during homeostasis, mainly via reactive oxygen species (ROS) and reactive metabolites such as formaldehyde and ethylene, and these are generally efficiently repaired. Exogenous DNA adducts can come from a variety of sources with exemplars being aflatoxin B1 (AFB1) produced by the fungus *Aspergillus flavus* which can thrive on poorly stored foodstuffs such as peanuts. Exposure produces the *N*7-Gua adduct that can induce GC→TA mutations, with increased risk of hepato carcinoma; 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), which is formed during the cooking of meat at high temperatures produces a *C*8-Gua adduct that can cause the GC→TA mutation and is linked to increased colon cancer risk; and tamoxifen, which is used to treat estrogen-receptor positive (ER+) breast cancer and is now approved as a chemopreventive, but it can produce *N*2-Gua adducts and is linked to elevated risk of endometrial cancer 27. These compounds produce bulky DNA adducts, cause specific mutations and are linked to an increased risk of cancer.

The aim of this study is to investigate whether acrylamide exposure from food as part of the normal diet leads to the production of DNA adducts that can be detected in human blood.

**Methods and materials:**

**Materials**

All chemicals were obtained from Sigma (Poole, UK).Optima LC-MS grade water, methanol and formic acid were obtained from ThermoFisher (Loughborough, UK). Stable isotope labelled [15*N*5]-2′-deoxyguanosine was obtained from Spectra Stable Isotopes, US. Blood & cell culture Genomic-tip 500/G DNA maxi kits and Proteinase K were purchased from Qiagen, (Manchester, UK). S-Monovette, K3 EDTA tubes used for blood collection were purchased from Sarstedt (Germany) for collecting the blood samples.

**Synthesis of *N*7-GA-Gua standard and internal standard**

Glycidamide was prepared by reaction of acrylonitrile with hydrogen peroxide28. The crude glycidamide preparation, with an estimated 20-fold molar excess, was reacted with 2′-deoxyguanosine in water or [15*N*5]-2′-deoxyguanosine dissolved in 50mM phosphate buffer, pH 7.0 at 60 °C for 4h 19. The resultant products were subjected to HPLC purification and confirmation of the identity of *N*7-GA-Gua was confirmed by NMR (Supplementary Information, Figure S1), MS and UV spectrometry, and [15*N*5]*N*7-GA-Gua was characterised by MS and obtaining UV scan spectra ranging between 240 and 320 nm. The concentration of the standards was calculated using the extinction coefficient (7.1 mM-1cm**-**1) by determining the UV absorbance at 285 nm 18.

**Sample collection**

Blood was collected (three tubes of 10mL) from healthy human volunteers using S-Monovette K3 EDTA tubes. The volunteers were recruited aged between 18 and 65, having no adverse medical conditions (including food allergies or intolerances) and not prescribed any medication other than contraception.

**Food frequency questionnaire**

Volunteers were recruited from staff and postgraduate/undergraduate students from the University of Leicester and asked to complete a FFQ for the 24 h period prior to blood sample donation. Ethics were obtained via the University of Leicester Ethics sub-committee for Medicine and Biological Sciences (Ethics reference: 18024-rgb6-ls:genetics&genomebiology). Information about the volunteers sex, weight, height, smoking status and alcohol intake was also obtained.

AA intake was estimated for each volunteer using the FFQ information by categorising each food/ drink item using the Food Standards Agency (FSA) report from 2019 2 and this was calculated as estimated AA intake (µg) per Kg reported bodyweight.

**DNA isolation**

DNA extraction from the human volunteer blood samples was performed using the Qiagen DNA extraction procedure. Each blood sample was transferred to a 50 mL tube and 9 mL of ice-cold C1 lysis buffer and 27 mL of ultrapure water were added and mixed gently by inversion to form a translucent suspension. The samples were kept on ice for 10 min before the lysed blood was centrifuged for 15 min at 1300 *g* and 4 °C and then the supernatant was discarded. The remaining pellet was re-suspended with 1.5 mL of ice-cold C1 buffer and 4.5 mL of ice-cold ultrapure water and was centrifuged for 15 min at 1300 g and 4°C, following removal of the supernatant the pellets were stored at -20°C until the next stage. The pelleted sample nuclei were dissolved in 5 mL of G2 buffer and two aliquots of the same sample were combined for incubation at 50 °C for 1h with the addition of 200 μL of Proteinase K (>600 mAU/mL). The samples were then centrifuged for 10 min at 5000 g and 4°C and applied to the Qiagen genomic-tip 500/G previously equilibrated with 10 mL of QBT buffer and allowed to elute by gravity flow. The genomic-tip was washed twice with 15 mL of QC buffer before the DNA sample was eluted with 15 mL of pre-warmed (at 50 °C) QF buffer. The eluate was collected and precipitated gently with the addition of 10.5 mL (0.7 volumes) of ice-cold isopropanol and centrifuged for 15 min at 5000 g and 4°C. The supernatant was then discarded and the DNA pellet was washed twice with 4 mL and then 1 mL of cold 70% ethanol/water (*v/v*).

The DNA pellet was then left to air-dry for 10 min and was resuspended with 500 μL of ultrapure water. The concentration was determined by measuring the absorbance at 260 nm using a Nanodrop UV spectrophotometer (ND-1000) following dilution with Tris-HCl buffer, pH 7.4 and assuming that one absorbance unit equals 50 g/mL for double stranded DNA. The isolated DNA solutions were stored at -20°C prior to hydrolysis and MS analysis.

**Sample preparation**

DNA samples (150µg) dissolved in LC-MS grade water were heated at 70°C for 1h using a heating block. The depurinated DNA samples were then filtered using 0.5mL 10K MWCO cut-off filters (Pierce™ Protein Concentrators from ThermoFisher, UK). Following filtration the samples were evaporated to dryness using a DNA speedvac (Savant SpeedvacPlus SC210A,ThermoFisher) and dissolved in 10 µL of 0.1% formic acid, containing 100 fmol/µL of the internal standard [15*N*5]*N*7-GA-Gua, then transferred to UPLC vials ready for analysis by LC-MS/MS.

**LC-ESI-MS/MS analysis of *N*7-GA-Gua**

The LC-MS/MS consisted of a Waters I Class UPLC system fitted with a 10 µL injection loop connected to a Xevo TQ-XS (Waters Ltd., Manchester, UK) tandem quadrupole mass spectrometer with an electrospray interface. The temperature of the electrospray source was maintained at 150ºC and the desolvation temperature at 350ºC. Nitrogen gas was used as the desolvation gas (600 L/h) and the cone gas was set to 150 L/h. The capillary voltage was set at 3.15 kV. The cone was set at 4 V. The mass spectrometer was tuned by using a *N*7-GA-Gua standard solution (5 pmol/µL) dissolved in 0.1% formic acid/methanol (95:5, *v/v*) introduced by continuous infusion at a flow rate of 5 µL/min using the XevoTQ-XS fluidics system.

A 5 µL aliquot (corresponding to 75 µg of depurinated DNA) of the sample was injected onto a Waters Acquity HSS T3 (2.1 × 100 mm, 1.8 µm) UPLC column. (Waters Ltd., Manchester, UK). The column was eluted isocratically 95:5, *v/v* with solvent A, 0.1% formic acid and solvent B, methanol at a flow rate of 0.2 mL/min with a run time of 10 min. The samples were analysed in positive electrospray ionization mode with collision induced dissociation MS/MS selected reaction monitoring (SRM) for the *N*7-GA-Gua adducted base [M+H]+ ion to guanine base [B+H2]+ quantifier ion transition of 239 to 152 *m/z* (20 eV) plus four qualifier ion transitions of 239 to 110 *m/z* (34 eV), 239 to 135 *m/z* (32 eV), 239 to 166 *m/z* (28eV) and 239 to 194 *m/z* (20 eV). Similarly for [15*N*5]*N*7-GA-Gua adducted base [M+H]+ ion to guanine base [B+H2]+ quantifier ion transition of 244 to 157 *m/z* (20 eV) and qualifier ion transitions of 244 to 199 *m/z* (20eV). The different collision energies for the various fragments ions monitored are shown in the brackets. The collision gas was argon (indicated cell pressure 3.0 × 10-3 mbar). The dwell time was set to 16 ms and the resolution was 0.75 Da at peak base. The level of *N*7-GA-Gua in each sample was determined from the ratio of the peak area of *N*7-GA-Gua to that of the internal standard [15*N*5]*N*7-GA-Gua.

**Calibration line for *N*7-GA-Gua**

The calibration line was constructed by analysing different amounts of the *N*7-GA-Gua (ranging from 0.5 to 2000 fmol detected on column) spiked with the internal standard [15*N*5]*N*7-GA-Gua (500 fmol detected on column). The intra- assay coefficients of variation of the LC-MS/MS method, were obtained following the analysis of 2.5, 25, and 250 fmol of *N*7-GA-Gua standards on column and the inter-assay coefficients of variation obtained on four different days.

**Results:**

**Synthesis:** The *N*7-GA-Gua adduct standard was synthesised (with a 30% yield) as described by Segerback *et al.* 19. Figure 2A shows a typical product ion spectrum obtained following collision induced dissociation MS/MS. The fragmentation of the *N*7-GA-Gua 239 *m/*z [M+H]+ ion resulted in product ions at 194 *m/*z, 152 *m/*z , 135 *m/*z and 88 *m/*z . The 88 *m/*z ion corresponds to fragmentation to glycidamide and 152 *m/*z results from fragmentation to guanine base [B+H2]+. The transition from 239 to 152 *m/z* was chosen as the SRM quantifier transition since the 152 *m/*z ion was the most abundant product ion. A similar product ion spectrum was obtained for the [15*N*5]*N*7-GA-Gua stable isotope labelled internal standard as shown in Figure 2B with the expected higher *m/z* for each guanine base fragment ion due to the presence of the [15*N*]-labelled nitrogen atoms. The SRM transition from 244 to 157 *m/z* was chosen as the quantifier transition for the internal standard.

**Validation of the LC-MS/MS method:**

A LC-MS/MS calibration line for the detection of *N*7-GA-Gua was obtained by analysing different amounts of the *N*7-GA-Gua standard spiked with a constant amount of the stable isotope internal standard [15*N*5]*N*7-GA-Gua (500 fmol). A linear response over the range from 0.5 to 2000 fmol was obtained for the detection of *N*7-GA-Gua with a linear regression correlation coefficient (r) of 0.999 (y = 0.0020x) as shown in Figure 2C. The limit of detection of the method was 0.25 fmol (S/N = 3.7), and the lower limit of quantitation was 0.50 fmol (S/N = 9.1). The intra- and inter-assay coefficients of variation of the method, which were obtained following the analysis of 2.5, 25, and 250 fmol of the *N*7-GA-Gua standard on column, are shown in Table 1.

Recovery experiments for the method were performed using calf thymus (ct) DNA as the matrix spiked with different amounts of the *N*7-GA-Gua standard. Following adjustment for background levels of *N*7-GA-Gua present in ctDNA the percentage recovery for *N*7-GA-Gua was found to be 113.1, 106.4 and 89.1% at 33, 166 and 333 fmol of *N*7-GA-Gua spiked into ctDNA, respectively (Supplementary Information, Figure S2, S3 and Table S1, S2).

**Volunteer participants**

Of the 24 volunteers recruited to the study, 17 samples provided the minimum amount of DNA required for the LC-MS/MS assay. The details of these volunteers are summarised in Table 2.

**Food frequency data**

The estimated mean AA intake was analysed by food group category, as described in the FSA report Hamlet *et al*, 2019 2 (Supplementary Information, Table S3). The biggest contributor was found to be potatoes, with roast and chipped (deep-fried) potatoes (cat. 10.5 and cat. 1) and potato crisps (cat. 2) accounting for over half of the AA, with 29.7% and 26.6% of the total intake, respectively. Baked goods such as bread (cat. 4), cake (cat. 10.2), and biscuits (cat. 6.3) account for 3.1%, 7.0% and 7.6% respectively. Cereals contribute (cat. 5) 9.3% and coffee contributes 7.0% (cat. 7). The remaining 9.7% contribution is from other products (cat. 10) which includes non-potato savoury snacks and chocolate (Figure 3).

**Sample preparation:**

The extracted human DNA samples were dissolved in LC-MS grade water and were heated at 70°C for 1h. The resulting depurinated DNA samples were then subjected to filtration using 10K MWCO cut-off filters to separate the *N*7-GA-Gua adduct from the unhydrolysed DNA backbone and then the filtrate was evaporated to dryness prior to analysis by LC-MS/MS. Samples were prepared for LC-MS/MS analysis by re-dissolving in 10 µL 0.1% formic acid containing 100 fmol/µL [15*N*5]*N*7-GA-Gua.

**Detection of *N*7-GA-Gua in human DNA**

The typical LC-MS/MS SRM ion chromatograms obtained for the determination of *N*7-GA-Gua adducts in a human blood DNA sample containing 500 fmol on column of the [15*N*5]*N*7-GA-Gua internal standard are shown in Figure 4A. For comparison Figure 4B shows the typical LC-MS/MS SRM ion chromatograms obtained for the solvent blank containing 500 fmol on column of the [15*N*5]*N*7-GA-Gua internal standard. The presence of a peak in each of the qualifier ion SRM transition channels (Figure 4A ii-v) provided conclusive confirmation that that *N*7-GA-Gua was being detected in the sample which co-eluted with the retention time of the [15*N*5]*N*7-GA-Gua peak. Any ionisation suppression due to the presence of the DNA matrix was negligible as evidenced by a matrix factor value of 98.8% which was calculated from the peak areas of [15*N*5]*N*7-GA-Gua spiked human DNA samples to that of the [15*N*5]*N*7-GA-Gua standard obtained following LC-MS/MS analysis.

**Discussion**

There is a large body of data suggesting that AA is carcinogenic in rodent models and that once ingested, AA is converted to the epoxide GA and can subsequently react with nucleic acid bases and form DNA adducts capable of initiating mutation.

A sensitive and selective LC-MS/MS method for the detection and quantitation of the *N*7-GA-Gua adduct a DNA adduct biomarker resulting from exposure to acrylamide has been developed and validated which uses a stable isotope labelled [15*N*5]*N*7-GA-Gua internal standard. This study has identified and quantified *N*7-GA-Gua from human blood samples, suggesting that dietary or environmental exposure to acrylamide has produced DNA adducts. The validated method has a lower limit of detection (LLOD) of 0.25 fmol (S/N = 3.7) and a lower limit of quantitation (LLOQ) of 0.50 fmol (S/N = 9.1), and out of 17 samples *N*7-GA-Gua was quantified in 13 from 75 µg DNA on-column, with the mean level of *N*7-GA-Gua being 4.61 fmol and the maximum 15.33 fmol, which equates to 1.90 adducts per 108 nucleotides, and 6.3 adducts per 108 nucleotides respectively (Figure 5). There was no direct correlation between estimated 24 h AA intake, based on the food frequency questionnaire (FFQ), and measured *N*7-GA-Gua levels in this volunteer study, which may not be surprising given the relative small sample size and inherent inaccuracies of FFQs and large, natural variation in AA levels in the same food/ drink category. These data are suggestive that dietary AA does produce DNA adducts, which in turn may cause DNA mutation, with the potential for initiating carcinogenesis. Currently, there is no evidence of increased cancer incidence linked to dietary AA in human populations, yet some regions of the world are implementing mitigation advice and benchmark levels to manufactures to reduce the amount of AA formed during food production 29. To investigate a link between dietary AA intake and measured *N*7-GA-Gua levels an alternative trial should be performed to include a cross-over study with controlled diets with high and negligible AA content, and genotyping for CYP2E1.

**Conflicts of interest**

All authors declare no conflict of interest

**Acknowledgements**

This work was supported by the World Cancer Research Fund (WCRF PhD studentship)

**Table 1**

The percentage intra- and inter-assay coefficients of variation for the LC-MS/MS detection of *N*7-GA-Gua.

|  |  |  |
| --- | --- | --- |
| *N*7-GA-Gua  (fmol on column) | Intra-day CV % (n = 4) | Inter-day CV % ( n = 4) |
| 2.5 | 1.5 | 2.5 |
| 25 | 1.8 | 3.6 |
| 250 | 0.9 | 2.9 |

**Table 2**

Study volunteer data

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Age (min, mean, max) | Weight (min, mean, max) | Sex | Smoker | AA intake (µg; min, mean, max) |
| 20, 34, 62 | 59, 69, 90 | F=10, M=7 | n = 1 | 20.0, 39.2, 78.6 |

**Figure 1**

Formation of acrylamide via the Miallard reaction between glucose and asparagine and the subsequent metabolism, detoxification and adduct formation of acrylamide and glycidamide.



Acrylamide (AA)

Asparagine

Glucose

Glycidamide (GA)

Glyceramide

CYP 2E1

Protein

Protein

DNA

GSH activation /

MA conjugation

Hb-Val-AA

Hb-Val-GA

*N*7-GA-Gua

AAMA

GAMA

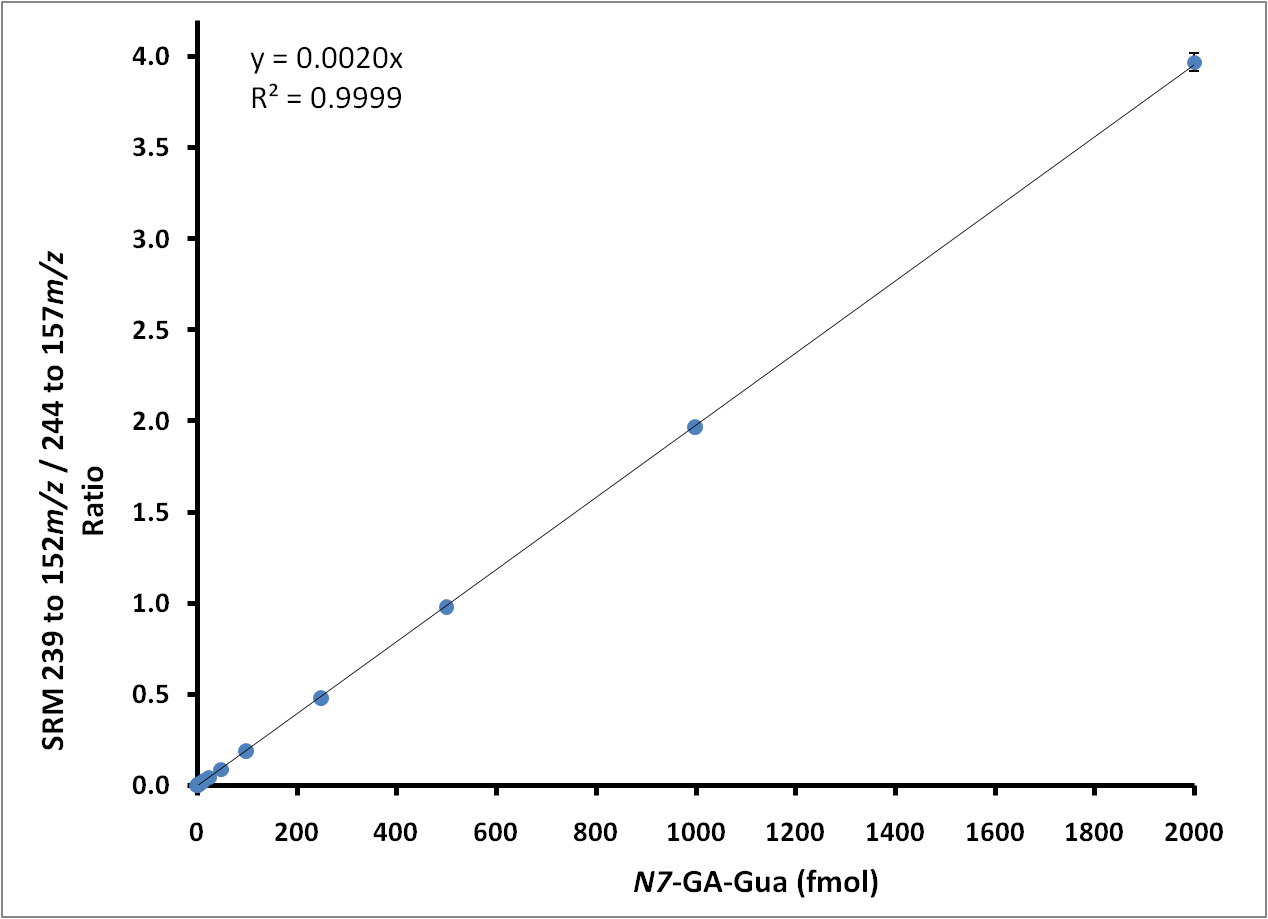
iso-GAMA

+

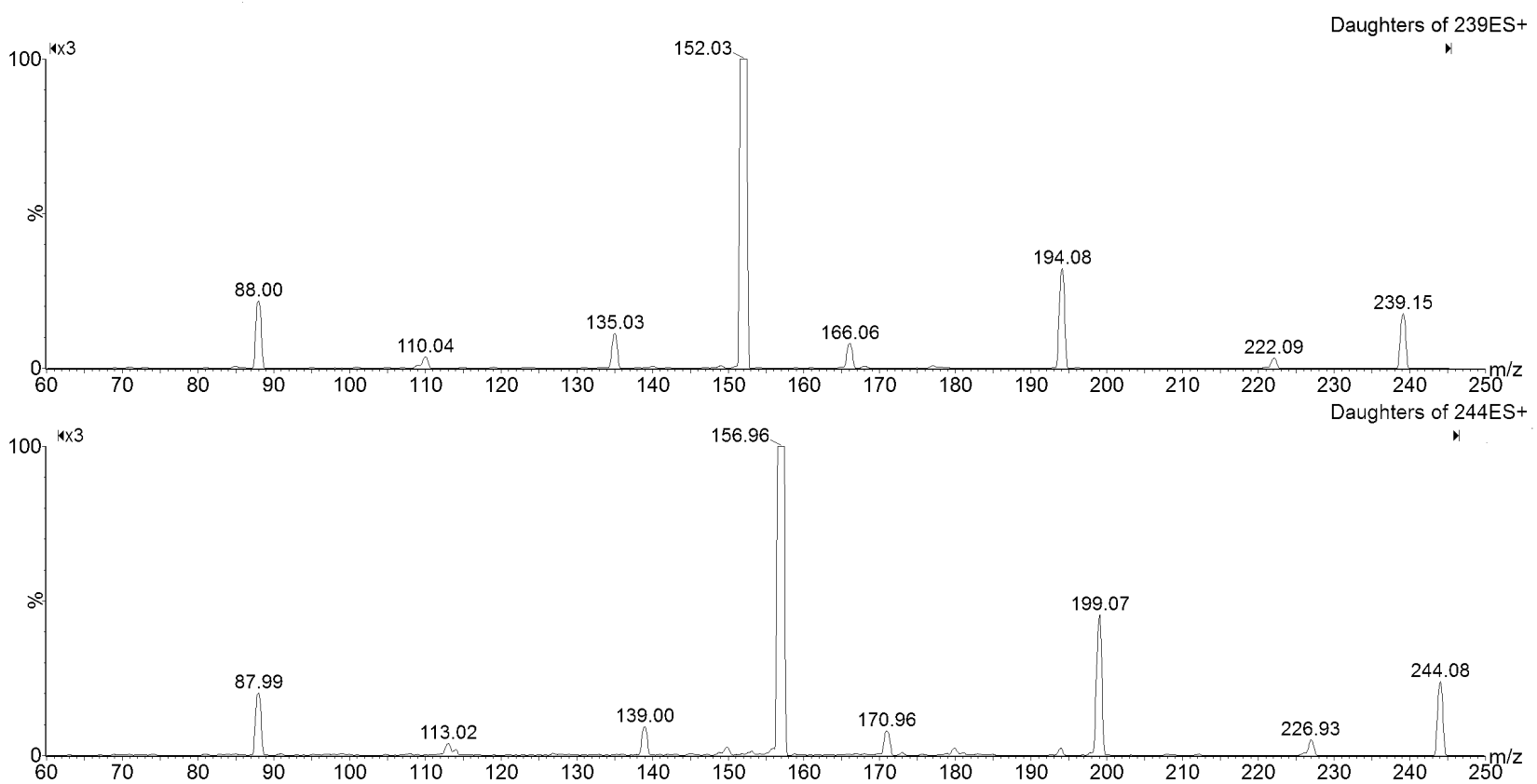
T > 120°C

**Figure 2**

Positive ESI-MS/MS collision induced dissociation product ion spectra for (**A**) *N*7-GA-Gua and (**B**) the stable isotope labelled internal standard [15*N*5]N7-GA-Gua. (**C**) LC-MS/MS calibration line for the detection of *N*7-GA-Gua.



**C**



**[15N5]*N7*-GA-Gua**

***N7*-GA-Gua**

**A**

**B**

**Figure 3**

Total estimated acrylamide intake diet contributions by food category

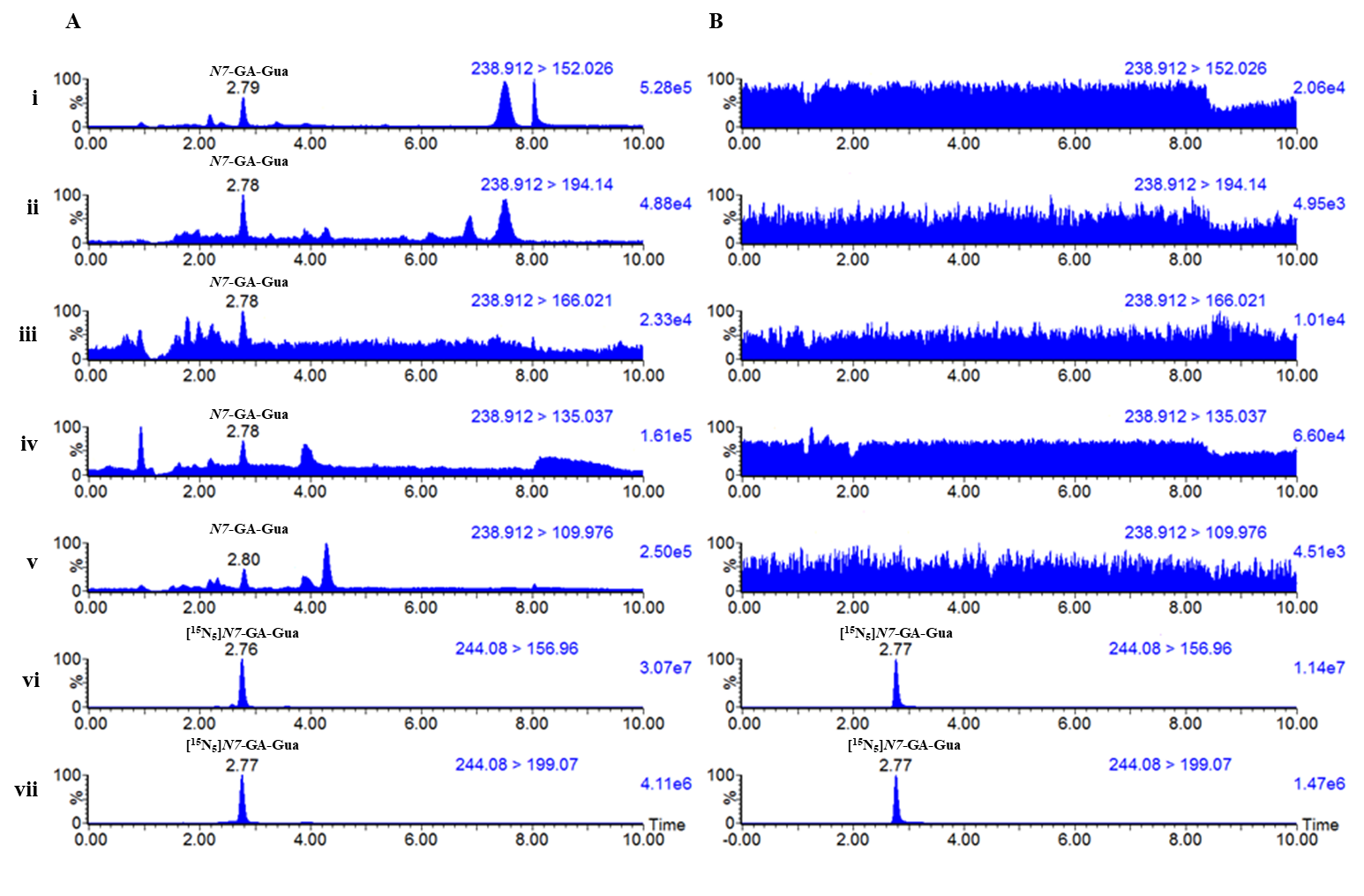
**Figure 4**

**A.** Typical LC-MS/MS SRM chromatograms for the determination of *N*7-GA-Gua in a human blood DNA sample containing 500 fmol internal standard, [15*N*5]*N*7-GA-Gua.

**B.** 0.1% formic acid solvent blank containing 500 fmol internal standard, [15*N*5]*N*7-GA-Gua.

The column was eluted isocratically 95:5, *v*/*v* with solvent A, 0.1% formic acid and solvent B, methanol at a flow rate of 0.2 mL/min.

**i)** 239→152 *m/z*, **ii**) 239→194 *m/z*, **iii**) 239→166 *m/z*, **iv**) 239→135 *m/z*, **v**) 239→109 *m/z*, **vi**) 244→157 *m/z*, **vii**) 244→199 *m/z*. **i**) quantifier transition for *N*7-GA-Gua, **ii – v**) qualifier transitions for *N*7-GA-Gua, **vi**) quantifier transition for [15*N*5]*N*7-GA-Gua, **vii**) qualifier transition for [15*N*5]*N*7-GA-Gua.



**Figure 5**

*N*7-GA-Gua levels measured in study volunteer DNA blood samples with estimated dietary AA intake 24 h prior to blood donation.



**References**

1. Reynolds T. Acrylamide and cancer: Tunnel leak in Sweden prompted studies. *J Natl Cancer Inst.* 2002;94(12):876-878.

2. Colin G. Hamlet LL, Ben Baxter, Donika Apostilova, Rozeela Ali. *Survey of Acrylamide and Furans in UK Retail Products: Summaries and Trends for Samples Purchased Between January 2014 and November 2018.* 2019.

3. Mojska H, Gielecinska I, Cendrowski A. Acrylamide content in cigarette mainstream smoke and estimation of exposure to acrylamide from tobacco smoke in Poland. *Ann Agr Env Med.* 2016;23(3):456-461.

4. Mottram DS, Wedzicha BL, Dodson AT. Acrylamide is formed in the Maillard reaction. *Nature.* 2002;419(6906):448-449.

5. Stadler RH, Blank I, Varga N, et al. Acrylamide from Maillard reaction products. *Nature.* 2002;419(6906):449-450.

6. Jezussek M, Schieberle P. A new LC/MS-method for the quantitation of acrylamide based on a stable isotope dilution assay and derivatization with 2-mercaptobenzoic acid. Comparison with two GC/MS methods. *J Agric Food Chem.* 2003;51(27):7866-7871.

7. Ono H, Chuda Y, Ohnishi-Kameyama M, et al. Analysis of acrylamide by LC-MS/MS and GC-MS in processed Japanese foods. *Food Addit Contam Part A-Chem.* 2003;20(3):215-220.

8. Rothweiler B, Kuhn E, Prest H. GC-MS approaches to the analysis of acrylamide in foods. *Am Lab.* 2004;36(12):35-+.

9. Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J Agric Food Chem.* 2002;50(17):4998-5006.

10. Pelucchi C, Bosetti C, Galeone C, La Vecchia C. Dietary acrylamide and cancer risk: An updated meta-analysis. *Int J Cancer.* 2015;136(12):2912-2922.

11. Sawada N, Iwasaki M, Yamaji T, et al. The Japan Public Health Center-based Prospective Study for the Next Generation (JPHC-NEXT): Study Design and Participants. *J Epidemiol.* 2020;30(1):46-54.

12. Ikeda S, Sobue T, Kitamura T, et al. Dietary Acrylamide Intake and the Risks of Renal Cell, Prostate, and Bladder Cancers: A Japan Public Health Center-Based Prospective Study. *Nutrients.* 2021;13(3):10.

13. Liu R, Sobue T, Kitamura T, et al. Dietary Acrylamide Intake and Risk of Esophageal, Gastric, and Colorectal Cancer: The Japan Public Health Center-Based Prospective Study. *Cancer Epidemiol Biomarkers Prev.* 2019;28(9):1461-1468.

14. Adani G, Filippini T, Wise LA, Halldorsson TI, Blaha L, Vinceti M. Dietary Intake of Acrylamide and Risk of Breast, Endometrial, and Ovarian Cancers: A Systematic Review and Dose-Response Meta-analysis. *Cancer Epidemiol Biomarkers Prev.* 2020;29(6):1095-1106.

15. Benford D, Ceccatelli S, Cottrill B, et al. Scientific Opinion on acrylamide in food. *Efsa J.* 2015;13(6):321.

16. Twaddle NC, Churchwell MI, McDaniel LP, Doerge DR. Autoclave sterilization produces acrylamide in rodent diets: Implications for toxicity testing. *J Agric Food Chem.* 2004;52(13):4344-4349.

17. Bailey E, Farmer PB, Bird I, Lamb JH, Peal JA. Monitoring exposure to acrylamide by the determination of S-(2-carboxyethyl)cysteine in hydrolyzed hemoglobin by gas-chromatography mass-spectrometry. *Anal Biochem.* 1986;157(2):241-248.

18. da Costa GG, Churchwell MI, Hamilton LP, et al. DNA adduct formation from acrylamide via conversion to glycidamide in adult and neonatal mice. *Chem Res Toxicol.* 2003;16(10):1328-1337.

19. Segerback D, Calleman CJ, Schroeder JL, Costa LG, Faustman EM. Formation of N-7-(2-carbamoyl-2-hydroxyethyl)guanine in DNA of the mouse and the rat following intraperitoneal administration of C-14 acrylamide. *Carcinogenesis.* 1995;16(5):1161-1165.

20. Shuker DEG, Farmer PB. Relevance of urinary DNA adducts as markers of carcinogen exposure. *Chem Res Toxicol.* 1992;5(4):450-460.

21. Farmer PB. DNA and protein adducts as markers of genotoxicity. *Toxicol Lett.* 2004;149(1-3):3-9.

22. Farmer PB, Brown K, Corcoran E, et al. DNA adducts: Mass spectrometry methods and future prospects. *Toxicol Appl Pharmacol.* 2004;197(3):164-164.

23. Gaskell M, Jukes R, Jones DJL, Martin EA, Farmer PB. Identification and characterization of (3 '',4 ''-dihydroxy)-1, N-2-benzetheno-2 '-deoxyguanosine 3 '-monophosphate, a novel DNA adduct formed by benzene metabolites. *Chem Res Toxicol.* 2002;15(8):1088-1095.

24. Huang CCJ, Wu CF, Shih WC, et al. Potential Association of Urinary N7-(2-Carbamoyl-2-hydroxyethyl) Guanine with Dietary Acrylamide Intake of Smokers and Nonsmokers. *Chem Res Toxicol.* 2015;28(1):43-50.

25. Huang YF, Huang CCJ, Lu CA, et al. Feasibility of using urinary N7-(2-carbamoy1-2-hydroxyethyl) Guanine as a biomarker for acrylamide exposed workers. *J Expo Sci Environ Epidemiol.* 2018;28(6):589-598.

26. Hemgesberg M, Stegmuller S, Cartus A, et al. Acrylamide-derived DNA adducts in human peripheral blood mononuclear cell DNA: Correlation with body mass. *Food and Chemical Toxicology.* 2021;157.

27. Hemminki K, Koskinen M, Rajaniemi H, Zhao CY. DNA adducts, mutations, and cancer 2000. *Regul Toxicol Pharmacol.* 2000;32(3):264-275.

28. Sugiyama S, Ohigashi S, Sawa R, Hayashi H. Selective preparation of 2,3-epoxypropanamide and its facile conversion to 2,3-dihydroxypropanamide with acidic resins. *Bull Chem Soc Jpn.* 1989;62(10):3202-3206.

29. Establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. 2021; <https://www.food.gov.uk/business-guidance/acrylamide-legislation>, 2021.