Quantitation of Four Guanine Oxidation Products from Reaction of DNA with Varying Doses of Peroxynitrite

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2. Calculation equations

(1) determination of the yield of DNA digestion

(2) determination of the artifacts of 8-oxodG

(3) determination of the artifacts of spiroiminodihydantoin

(4) determination of the artifacts of guanidinohydantoin

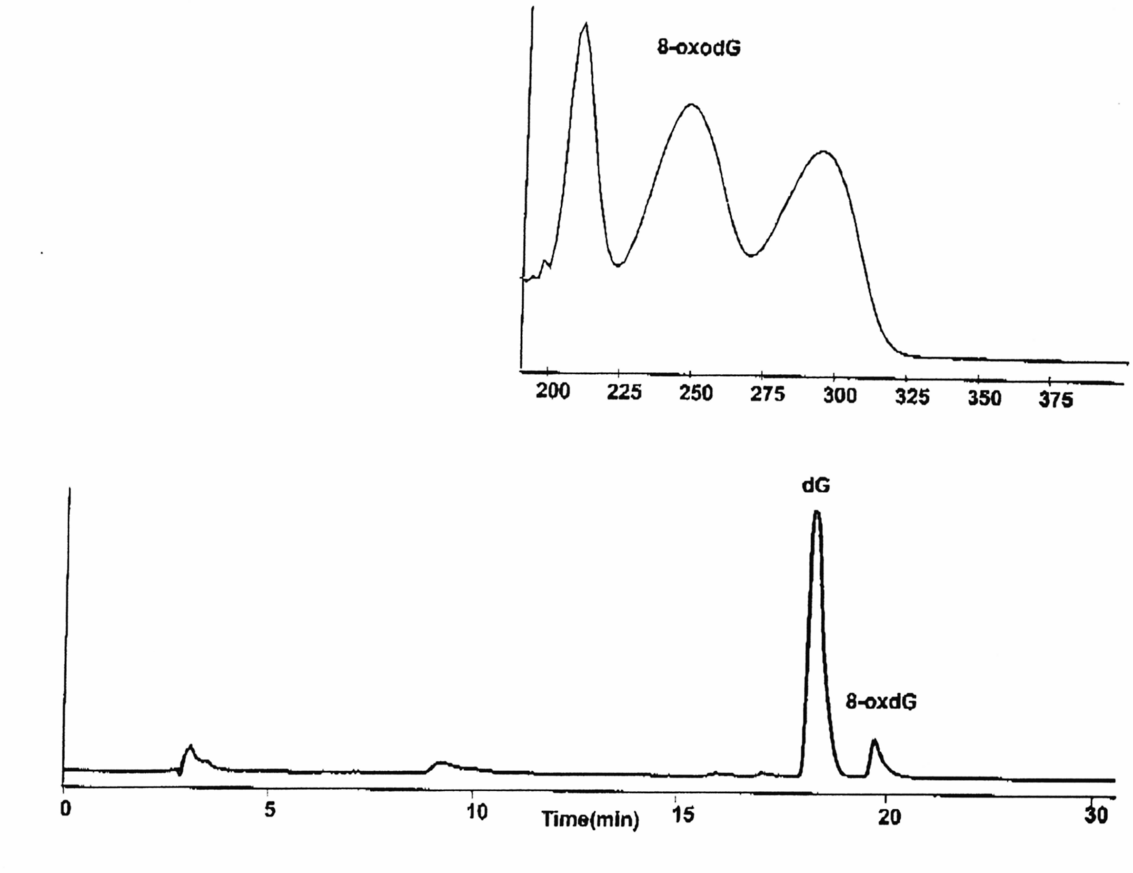

Figure 1 HPLC chromatogram of purification of 8-oxodG from oxidation of dG nucleoside

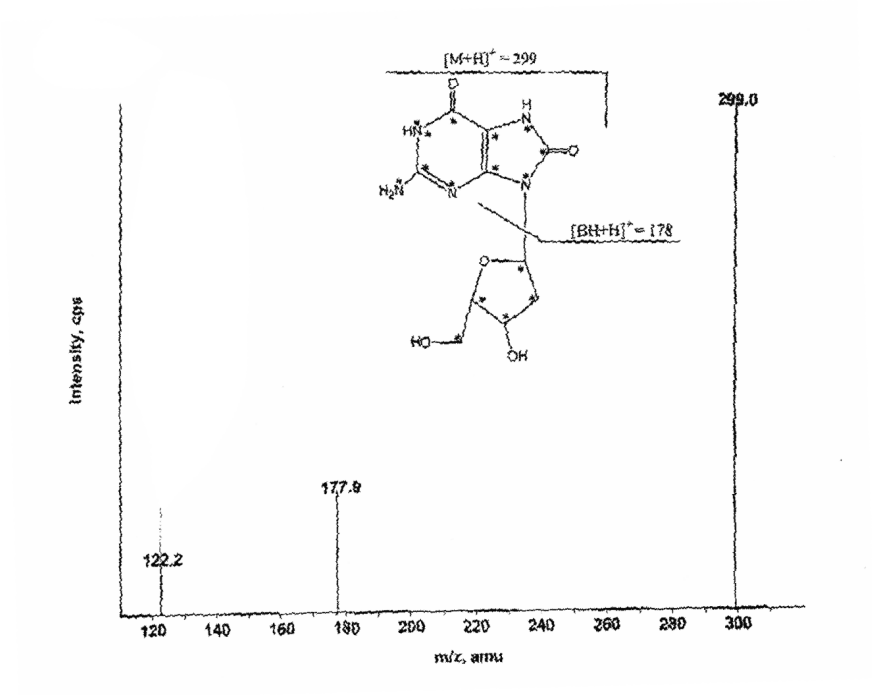

Figure 2 ESI-MS of the uniformly ^{13}C, ^{15}N labeled 8-oxodG standard

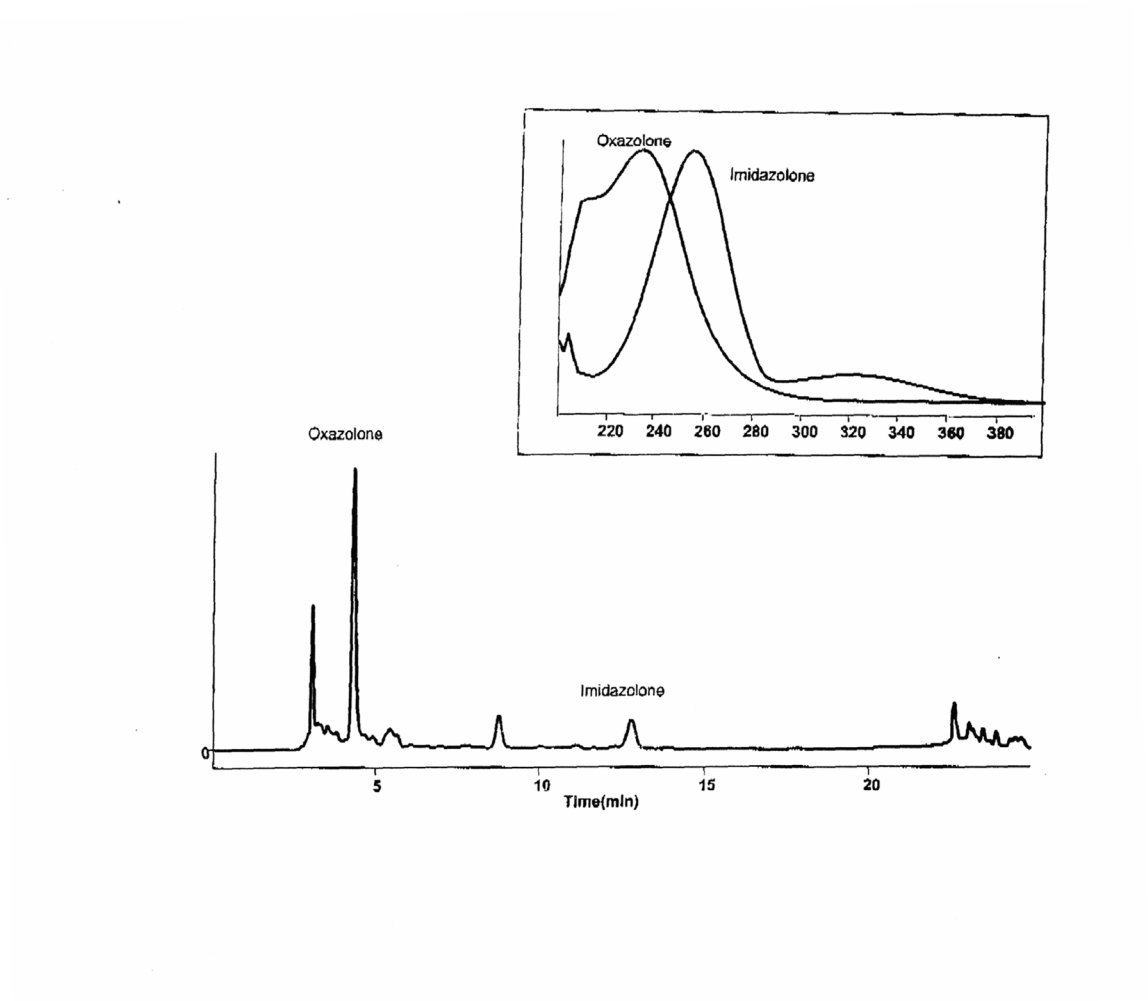

Figure 3 HPLC Chromatogram of converting imidazolone nucleoside to oxazolone nucleoside

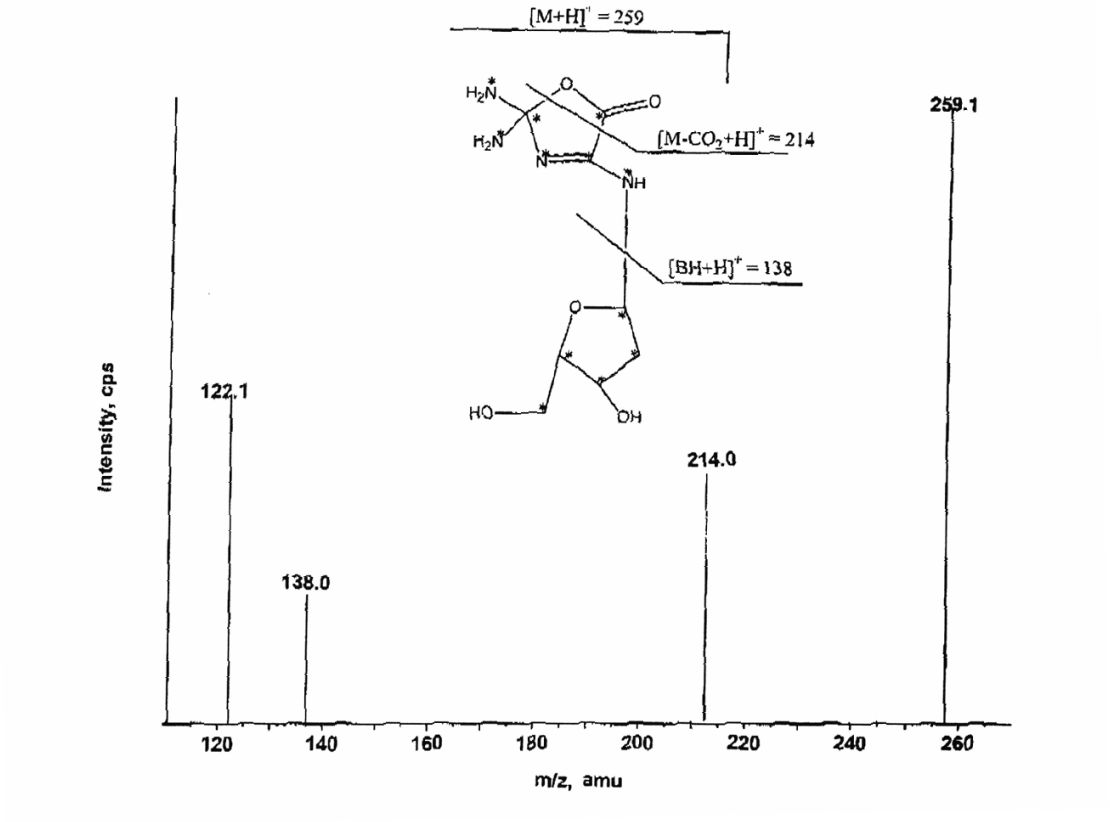

Figure 4 ESI-MS of the uniformly ^{13}C, ^{15}N labeled oxazolone standard

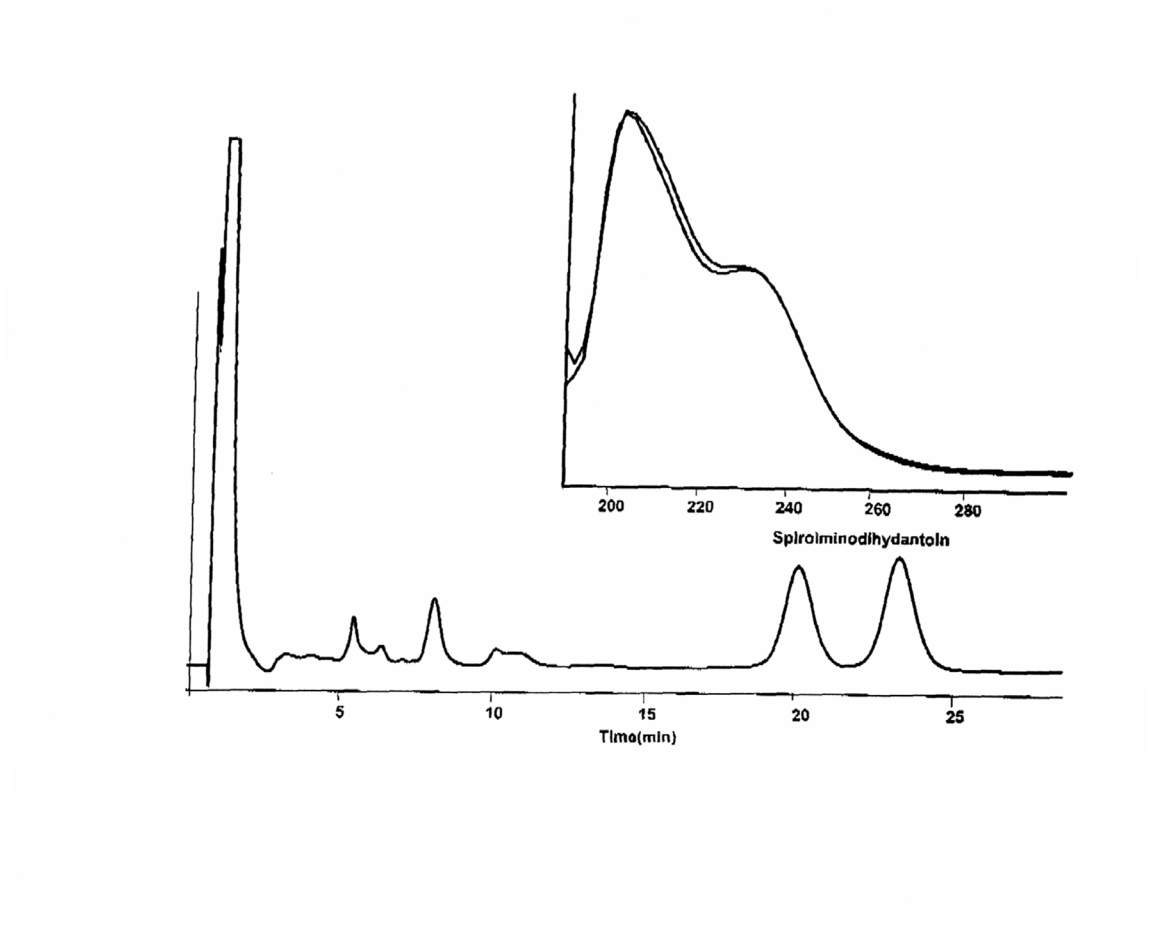

Figure 5 HPLC chromatogram of purification of spiroiminodihydantoin standard from dC, dT, dG and dA nucleosides

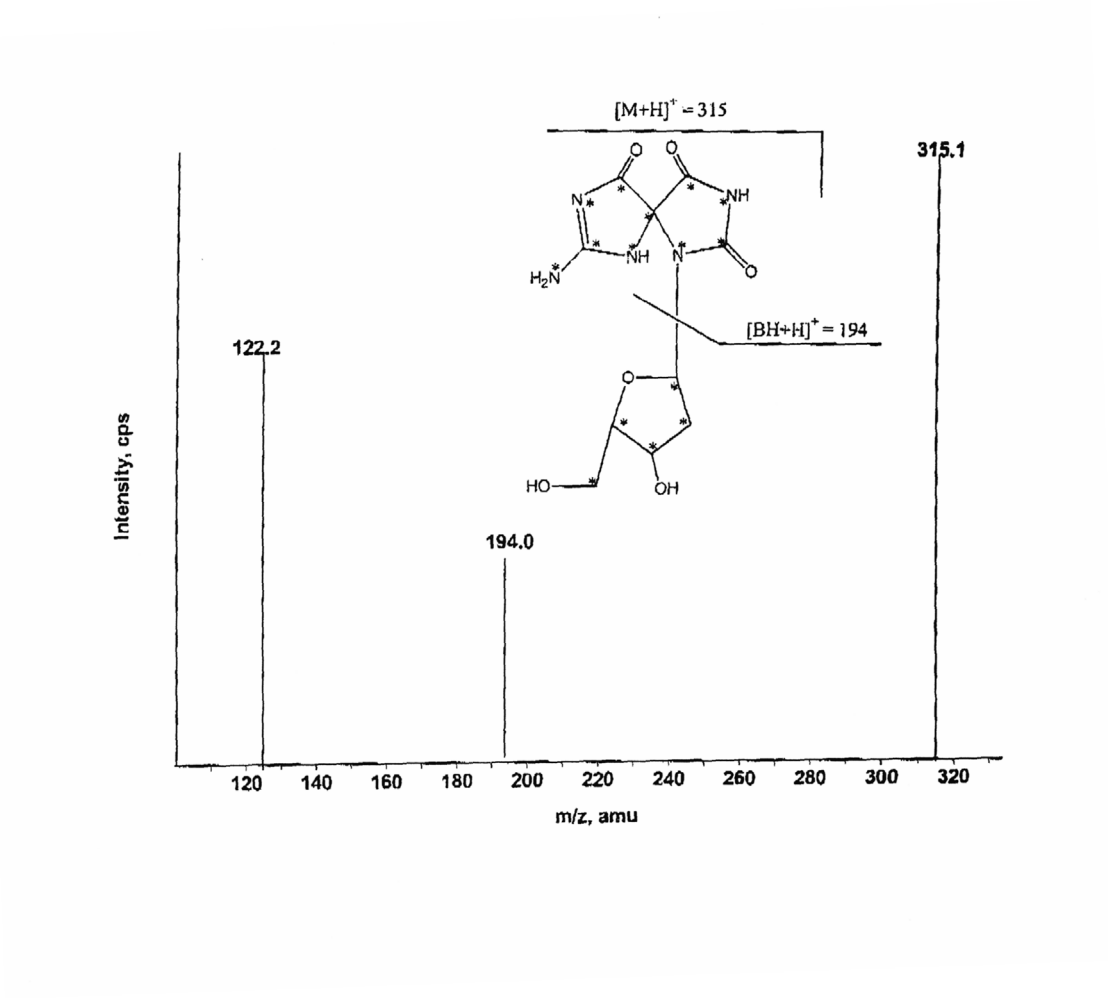

Figure 6 ESI-MS of the uniformly ^{13}C, ^{15}N labeled spiroiminodihydantoin standard

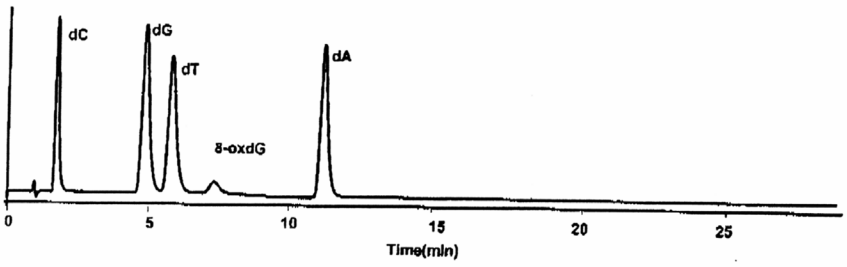

Figure 7 HPLC chromatogram of purification of 8-oxodG standard from dC, dT, dG and dA nucleosides

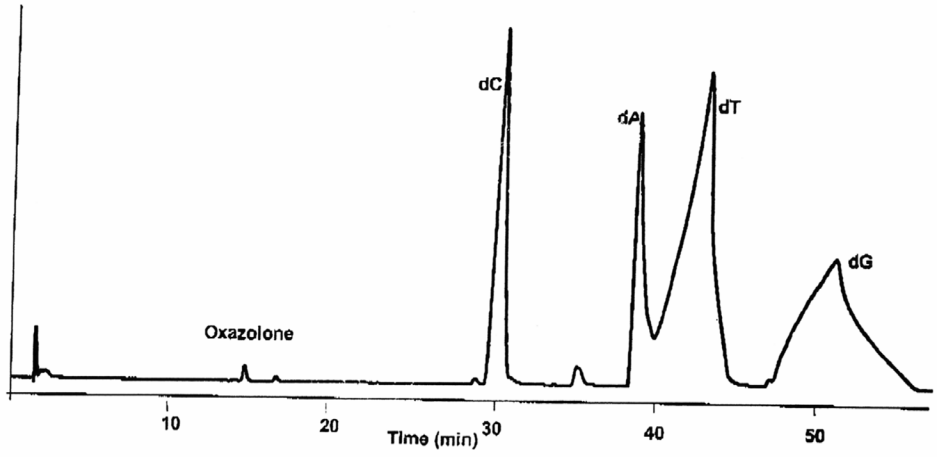

Figure 8 Purification of oxazolone standard from untreated DNA digestion mixture

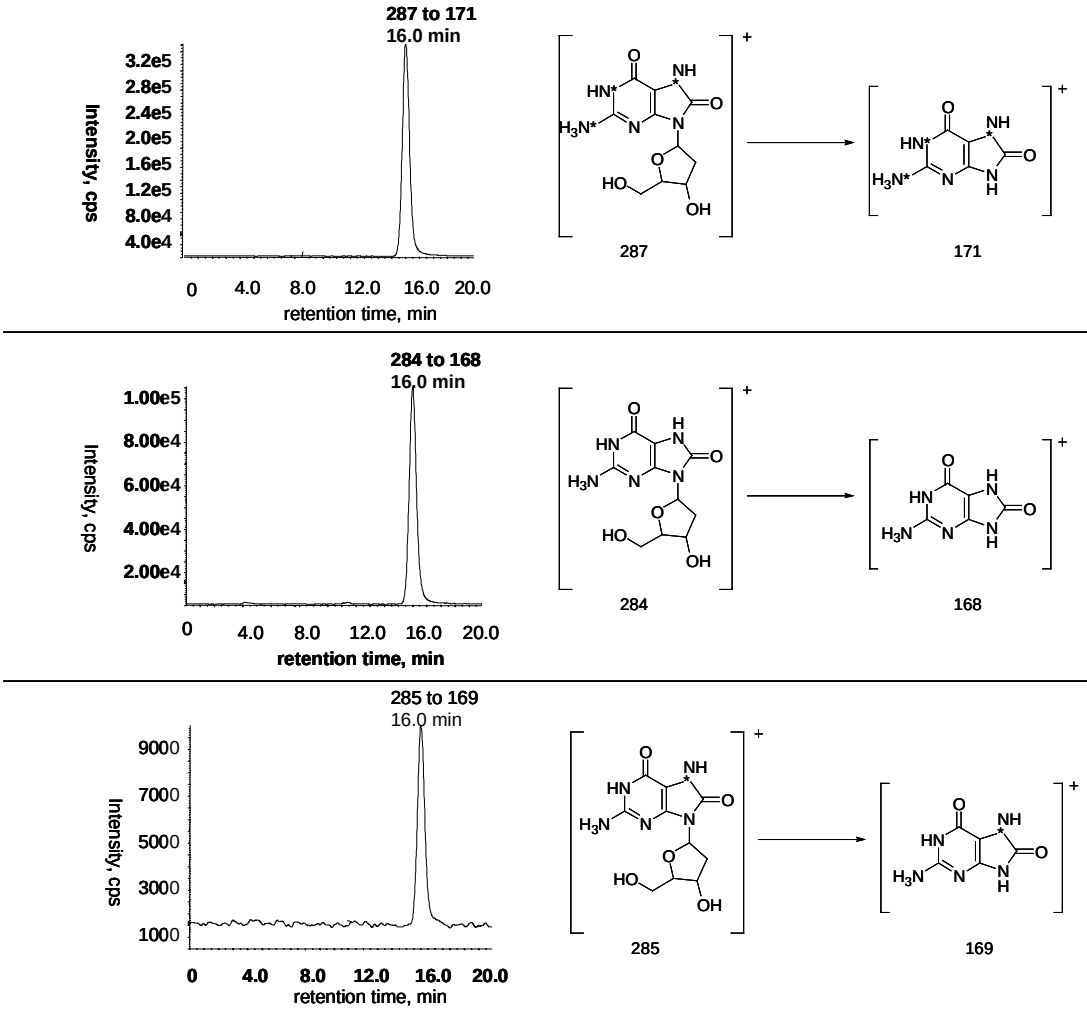

Figure 9 MRM chromatograms of detection of 8-oxodG in untreated DNA by LC-MS/MS in the presence of 7-^{15}N-dG

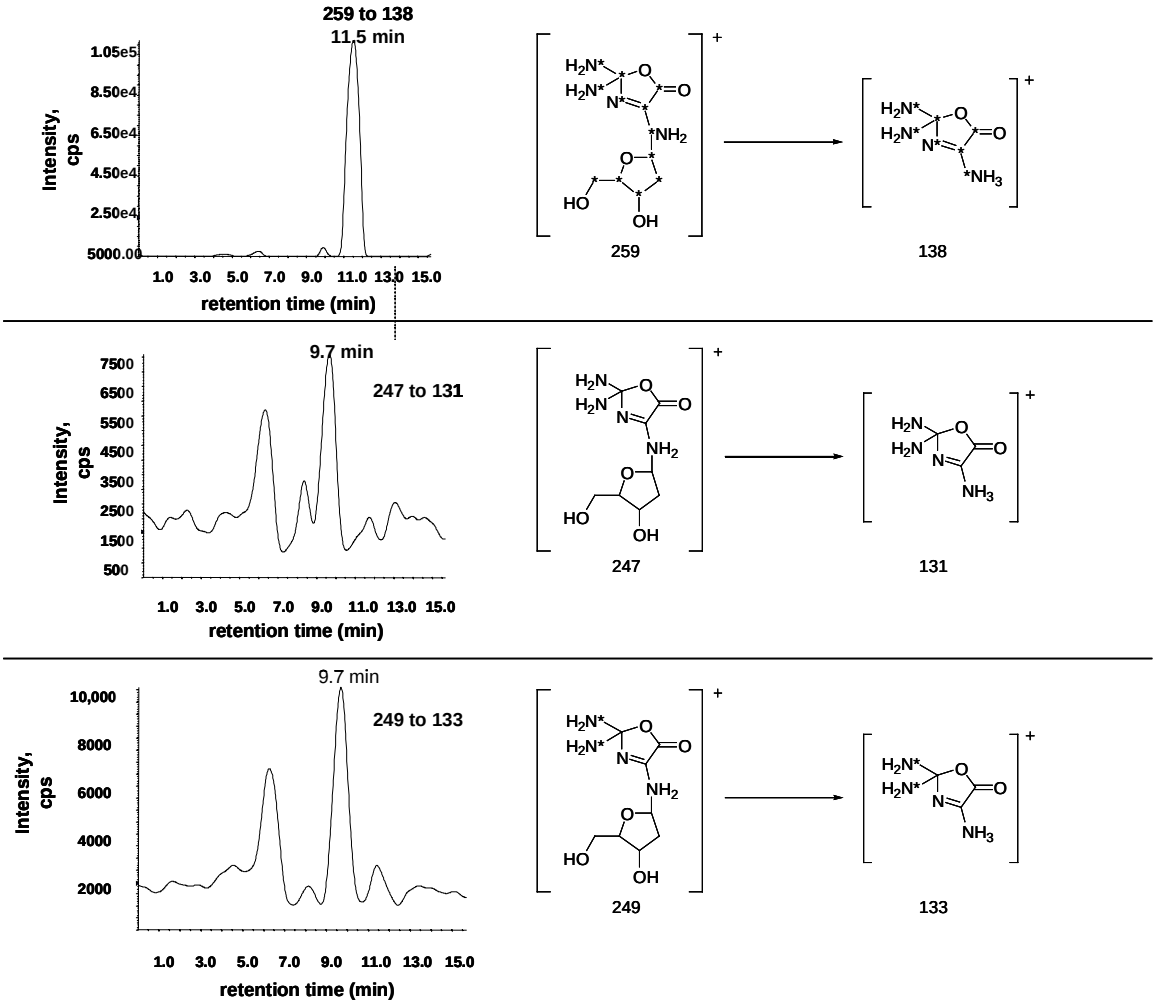

Figure 10 MRM chromatograms of attempt to detect oxazolone in untreated DNA by LC-MS/MS in the presence of 1,2,7-$^{15}N_3$-8-oxodG. No oxazolone was detected in untreated DNA.

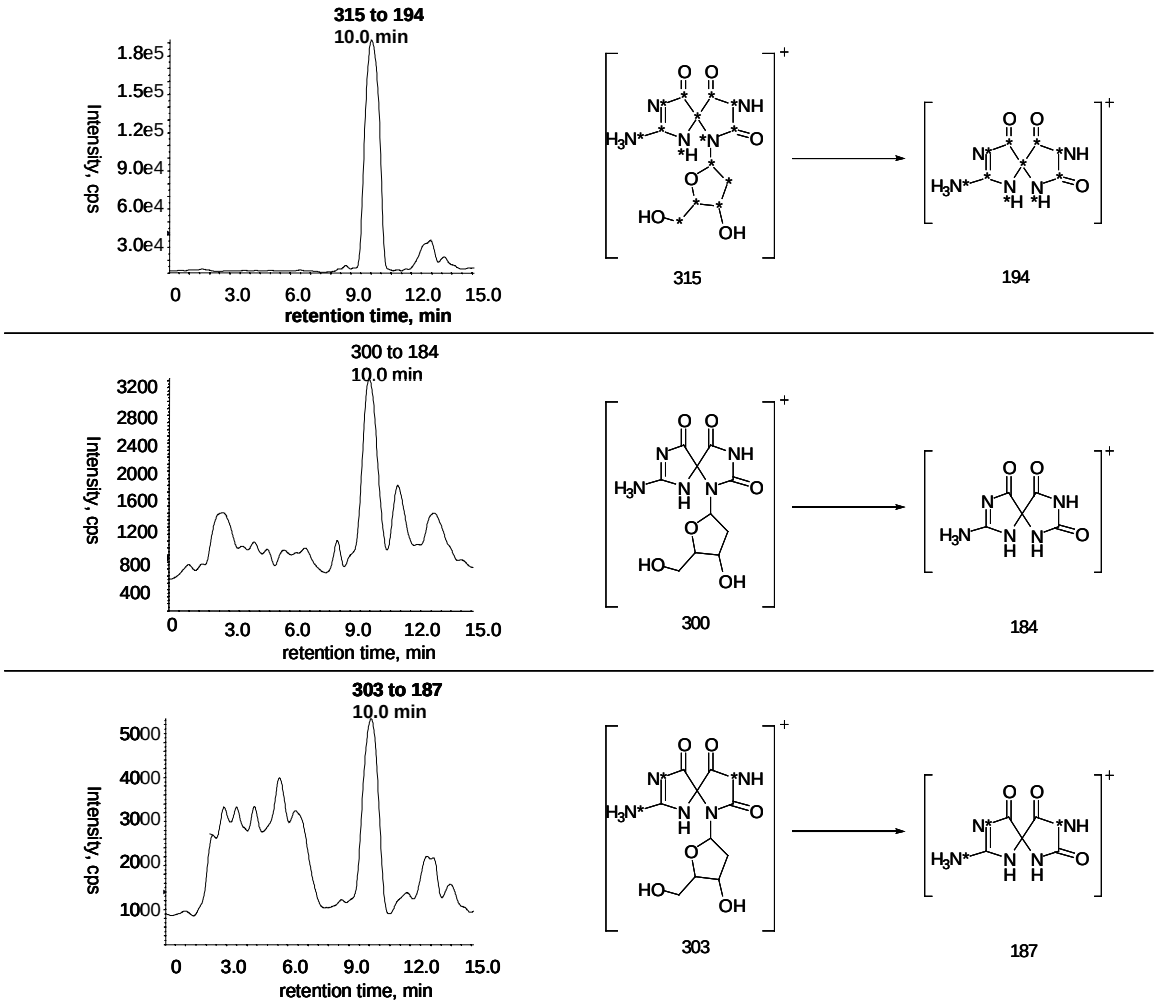

Figure 11 MRM chromatograms of detection of spiroiminodihydantoin in untreated DNA by LC-MS/MS on a

capillary C18 column in the presence of 1,2,7-$^{15}N_3$-8-oxodG.

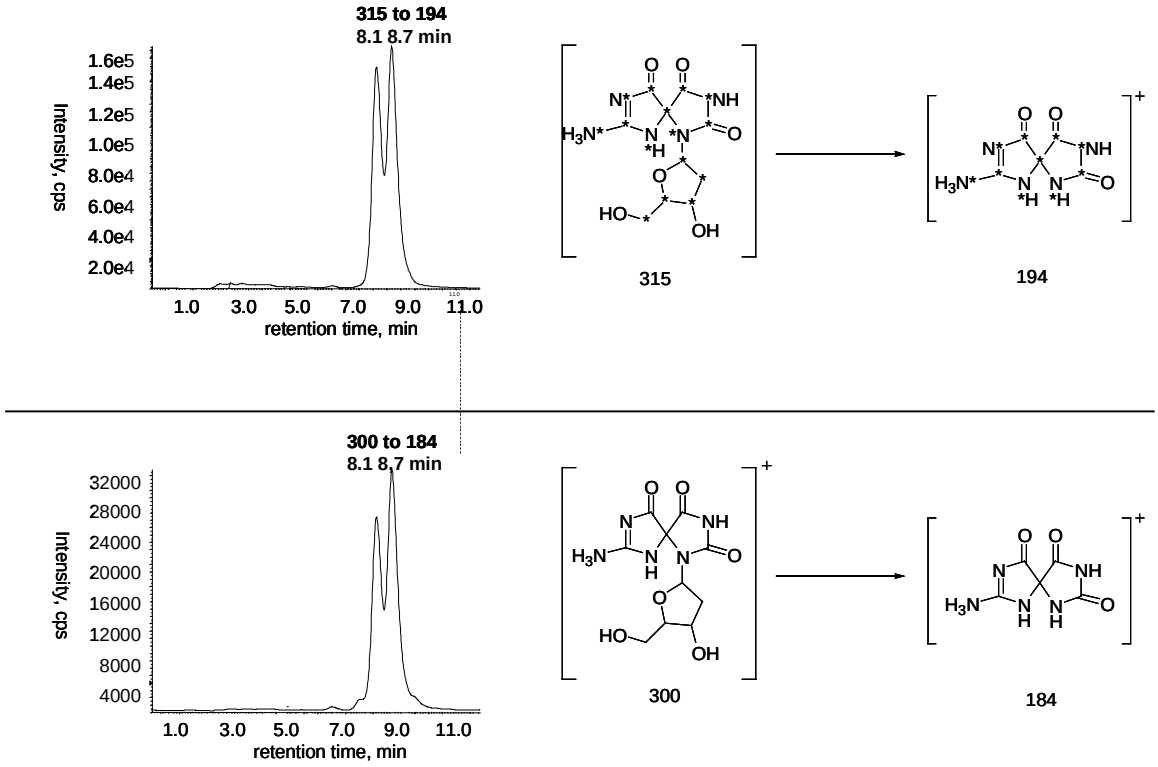

Figure 12 MRM chromatograms of detection of spiroiminodihydantoin in untreated DNA by LC-MS/MS on a capillary hypercarb column

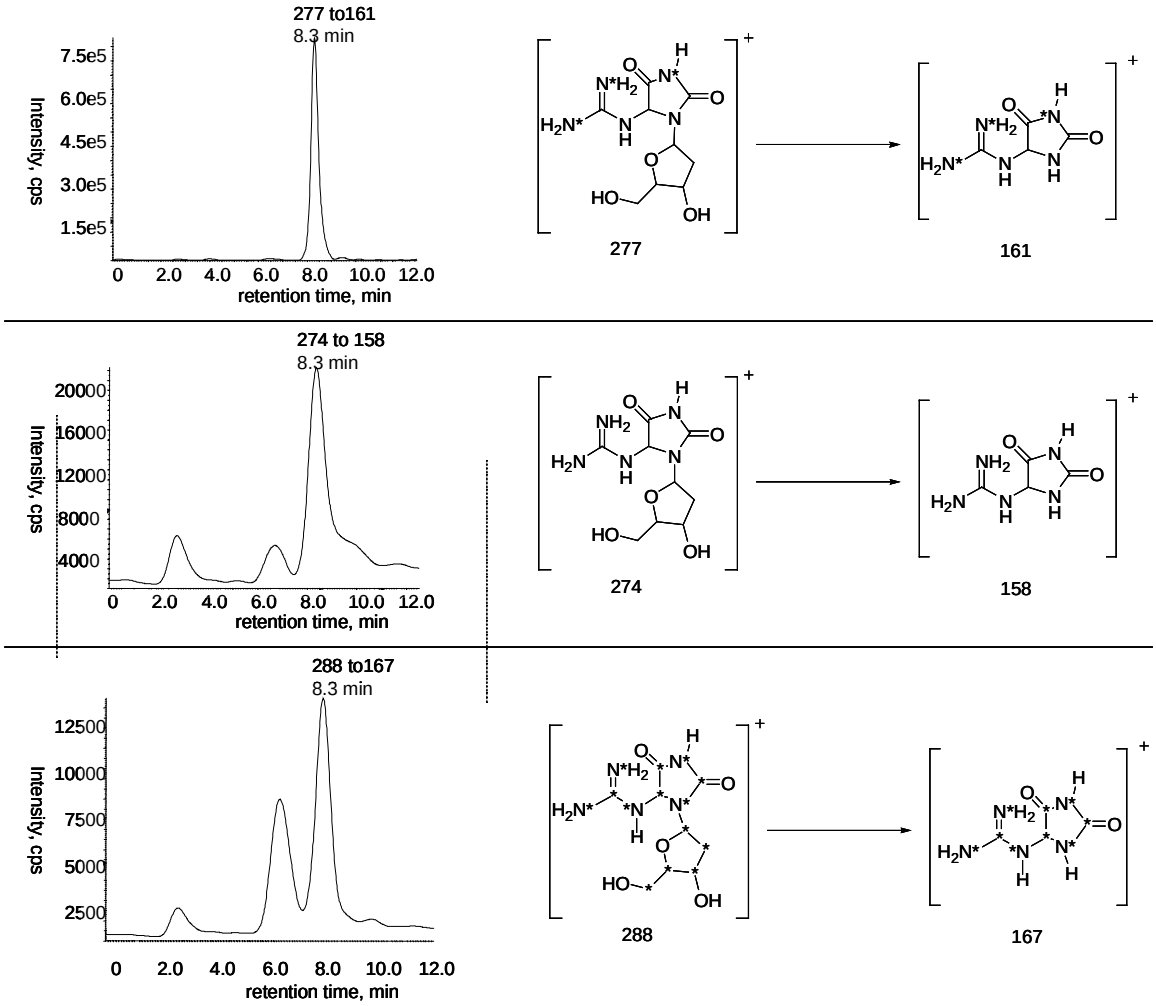

Figure 13 MRM chromatograms of detection of guanidinohydantoin in untreated DNA by LC-MS/MS in the presence of uniformly ^{13}C, ^{15}N labeled 8-oxodG.

Calculation Equations

1. Calculation of the yields of DNA digestion
   (1) dG from DNA digestion: dG (mol) = ^{15}NdG (mol) * response ratio(dG/^{15}NdG)
   (2) molar ratio between dG and ^{15}NdG = dG(mol)/ ^{15}NdG (mol)
   (3) theoretical dG: dG (mol) = DNA(g)/(average nucleotide M.W, 331 g/mol)/4
   (4) yield of digestion = dG from digestion (mol)/ dG from theoretical calculation (mol)

2. Calculation of the artifacts of 8-oxodG from spurious oxidation of dG
   MRM signal of 8-oxodG labeled internal standard = A
   MRM signal of 8-oxodG = B
   MRM signal of 7-^{15}N-8-oxodG = C
   Molar ratio between dg and ^{15}NdG = D
   Labeled 8-oxodG internal standard (mol) = E

   7-^{15}N-8-oxodG (mol) = C/A * E (assumption: the response ratio between 7-^{15}N-8-oxodG and labeled 8-oxodG internal standard = 1)

   8-oxodG (mol) = B/A* response ratio (8-oxodG/8-oxodG internal standard) * E

   artifacts of 8-oxodG (mol) = 7-^{15}N-8-oxodG (mol) * D

   corrected 8-oxodG (mol) = 8-oxodG (mol) – artifacts of 8oxodG (mol)

3. Calculation of the artifacts of spiroiminodihydantoin from spurious oxidation of 8-oxodG
   MRM signal of spiro labeled internal standard = A
   MRM signal of spiro = B
   MRM signal of $^{15}N_3$-spiro = C
   8-oxodG (mol) = D
   1,2,7-^{15}N-8-oxodG (mol) = E
   labeled spiro internal standard (mol) = F
   mol% of oxidation of 1,2,7-^{15}N-8-oxodG to $^{15}N_3$-spiro = G

   spiro (mol) = B/A* response ratio (spiro/spiro internal standard) * F

   $^{15}N_3$-spiro (mol) = C/A* F (assumption: the response ratio between $^{15}N_3$-spiro and labeled spiro internal standard =1)

   G = $^{15}N_3$-spiro (mol)/E *100

   Artifacts of spiro (mol) = G * D

   Corrected spiro (mol) = spiro (mol) – artifacts of spiro (mol)

4. Calculation of the artifacts of guanidinohydantoin from spurious oxidation of 8-oxodG
   MRM signal of guanidino labeled internal standard = A
   MRM signal of guanidino = B
   MRM signal of uniformly ^{13}C, ^{15}N labeled guanidino = C
   8-oxodG (mol) = D
   uniformly ^{13}C, ^{15}N labeled 8-oxodG (mol) = E
   labeled guanidino internal standard (mol) = F
   mol% of oxidation of uniformly ^{13}C, ^{15}N labeled 8-oxodG to uniformly ^{13}C, ^{15}N labeled guanidino = G

guanidino (mol) = B/A* response ratio (guanidino/guanidino internal standard) * F

uniformly ^{13}C, ^{15}N labeled guanidino (mol) = C/A* F (assumption: the response ratio between uniformly ^{13}C, ^{15}N labeled guanidino and labeled guanidino internal standard =1)

G = uniformly ^{13}C, ^{15}N labeled guanidino (mol)/E *100

Artifacts of guanidino (mol) = G * D

Corrected guanidino (mol) = guanidino (mol) – artifacts of guanidino (mol)