

Selective Digestion and Novel Cleanup Techniques for Detection of Benzo[a]pyrene-DNA Adducts by CE/MS

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Introduction: Much research has focused on the low level detection of DNA adducts as a possible measure of exposure to certain carcinogens and cancer risk assessment. Benzo[a]pyrene (BaP) is a ubiquitous environmental PAH and has been found to covalently attach to DNA. Previously in our laboratory, BaP-derived adduct detection was achieved by enzymatically reducing DNA to individual nucleotides, followed by liquid-liquid extraction or solid-phase extraction (SPE) for isolation of adducted nucleotides.¹ These DNA adducts were subsequently analyzed by CE/MS. These cleanup methods, however, are labor-intensive and require the samples to be dissolved in large volumes. The following research will focus on improved sample-cleanup techniques for the sensitive detection of BaP-nucleotide adducts by CE/MS.

Experimental: *In vitro* DNA samples were prepared by incubating DNA with the active form of BaP, benzo[a]pyrene-diol epoxide (BPDE). Following incubation, DNA was digested using micrococcal nuclease, spleen phosphodiesterase and nuclease P1. SPE was performed using Millipore ZipTips containing an immobilized metal affinity resin, and were charged with ferric chloride prior to use. Retained nucleotides and BaP-adducted nucleotides were released via basic conditions. CE/MS was performed using 10 mM ammonium acetate (pH 9), +15 kV and a 75 cm x 75 μ m capillary. UV detection was performed at 279 nm and MS detection was achieved using a TSQ 700 triple quadrupole instrument (Full-scan, SIM and MRM modes). CE/MS was performed using a coaxial sheath liquid consisting of 70:30 methanol:CE buffer. For adduct analyses, the poor concentration detection limits of CE (due to nL injection volumes) were not suitable for the detection of low-level adducts. Therefore, sample stacking was employed by hydrodynamic loading for 5-20 minutes and reversing the CE voltage to -15 kV. As the current approached the value obtained by standard CE (buffer-filled), the polarity was returned to +15 kV for analysis. This methodology allowed significantly higher sample loading capacity and subsequent increase in detection limit.

Results and Discussion: Due to the unique chemical structure of BaP, the enzyme nuclease P1 is unable to remove the phosphate moiety from adducted nucleotides. Therefore, metal affinity SPE is used to selectively extract phosphorylated components (ie. adducted nucleotides and undigested normal nucleotides). Initial CE/UV method development was performed with a standard solution of dG nucleosides and dGp nucleotides (to mimic dGp-BPDE). Figure 1 demonstrates the ability of metal affinity ZipTips for the enrichment of nucleotides without retaining any detectable nucleosides (UV detection at 279 nm). Nucleotides could be effectively extracted even in the presence of 1,000,000x the concentration of normal nucleosides, which makes this method well suited for extracting adducted nucleotides from DNA digestion samples. Due to the low concentration detection limit of CE, sample stacking was employed for detection of *in vitro* formed adducts. Figure 2 shows the CE/MS analysis of SPE-cleaned *in vitro* formed dGp-BPDE in full-scan mode. In addition to dGp-BPDE, undigested normal nucleotides can also be seen due to incomplete digestion. The CE/MS/MS spectrum of dGp-BPDE is shown in Figure 3. To increase detection limits, the triple quadrupole mass spectrometer was also used to detect adducts in the SIM (not shown) and SRM modes (Figure 4). Although SIM detection displayed a S/N of almost 400 for the same sample, SRM detection offers the additional advantage of selectivity. The transition 648->195 was used to detect the BaP adduct.

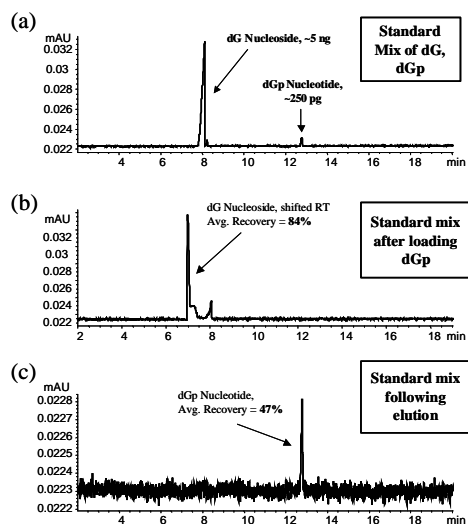


Figure 1: Extraction efficiency of dGp

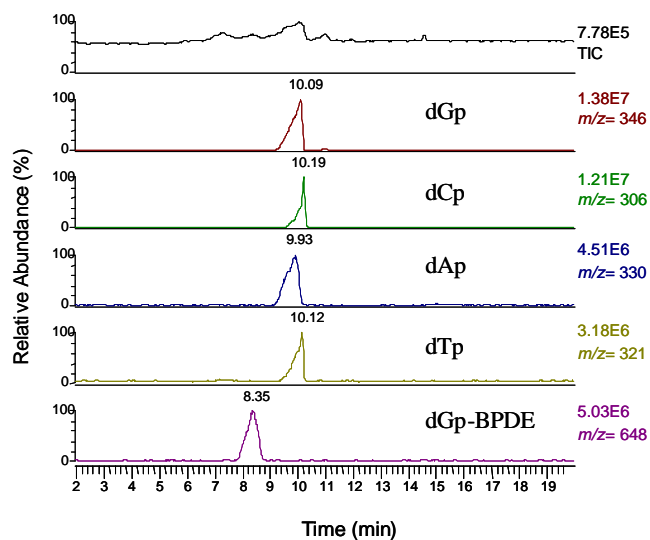


Figure 2: CE/MS (full-scan) of *in vitro* digest

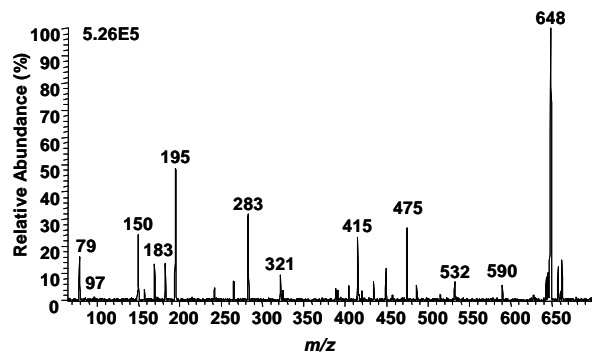


Figure 3: CE/MS/MS of dGp-BPDE

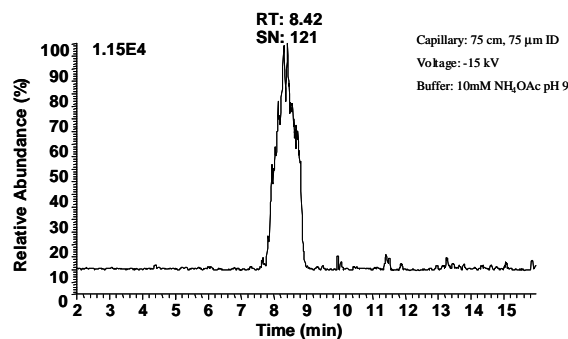


Figure 4: CE/MS (SRM) of dGp-BPDE

Conclusions: The work presented here describes an alternative method for the digestion and cleanup of *in vitro* carcinogen-DNA adducts, followed by CE/MS analysis. This method has several advantages over previously described SPE cleanup performed in our laboratory¹ and others² for dGp-BPDE adducts:

- Fast
- Simple
- Commercially available
- No need for excessive dilution (small scale)
- Ability to use with automation
- Possible applicability to select nucleotide adducts

For these reasons, this technique is extremely promising for the cleanup of *in vivo* formed BPDE adduct samples prior to separation and low-level MS detection.

References

1. Barry, J. P.; Norwood, C.; Vouros, P. *Anal Chem* **1996**, *68*, 1432.
2. Willems, A. V.; Deforce, D. L.; Van Den Eeckhout, E.G.; Lambert, W. E.; Van Peteghem, C. H.; De Leenheer, A. P.; Van Bocxlaer, J.F. *Electrophoresis* **2002**, *23*, 4092.