

Detection and Quantification of dG-C8-ABP DNA Adducts in Human Pancreas Samples using Capillary Liquid Chromatography/Microelectrospray/Mass Spectrometry

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Introduction: Cigarette smoking has long been associated with numerous cancers, including lung, larynx, pharynx, and esophagus. Research has shown that various constituents present within cigarette smoke are carcinogenic. One group of compounds particularly potent to rodents is aromatic amines (AA), with the most potent group member being 4-aminobiphenyl (4-ABP). Through prolonged exposure, these compounds have already been linked to tumor formation in various animal models. 4-Aminobiphenyl, present in nanogram quantities in cigarette smoke, can be metabolically activated to N-acetoxy-4-aminobiphenyl, which binds covalently to DNA. Our laboratory has developed an LC-MS method for the quantification and detection of dG-C8-ABP in human pancreatic tissue. Figure 1, shows the known structure of dG-C8-ABP.

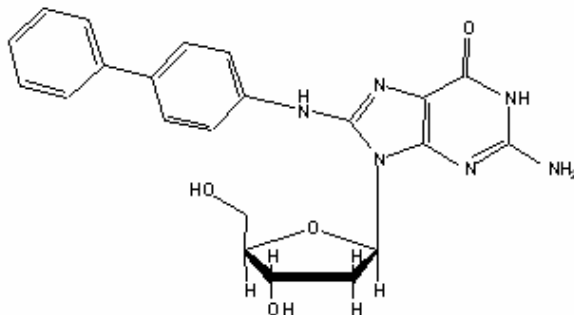


Figure 1: Structure of dG-C8-ABP

Methods: Adduct standards of dG-C8-ABP and internal standard, dG-C8-ABP-*D*₉ were synthesized by reaction of N-acetoxy-4-aminobiphenyl with 2'-deoxyguanosine. Standard curves were prepared by addition of both standard and internal standard adducts to calf thymus DNA. The calf thymus DNA was then enzymatically digested to nucleosides and adducts were isolated from normal nucleosides by C₁₈ solid phase extraction. DNA was isolated from human pancreas tissue and was processed in the same manner as the standard curves in order to isolate adducted nucleosides. Samples were quantified for dG-C8-ABP adducts using reverse phase capillary liquid chromatography/electrospray mass spectrometry. Chromatographic separations were performed on a Beta Basic, C₁₈ capillary column, 0.32 mm ID, 50mm length, 3 μm particle size. Mass spectral analyses were performed using a TSQ Quantum AM triple quadrupole mass spectrometer equipped with a microspray interface.

Results and Discussion: Mass spectral analyses began with an infusion of N-(deoxyguanosine-8-yl)-4-aminobiphenyl (dG-C8-ABP) and the deuterated internal standard, dG-C8-ABP-*D*₉ showing [M+H]⁺ peaks at *m/z* 435 and 444, respectively. Increasing the collision voltage gave peaks at 319 and 328, respectively indicating the characteristic loss of the sugar moiety from the nucleoside. Recognition of these patterns enabled the development of a comprehensive LC/MS trace level analysis using selected reaction monitoring. Capillary liquid chromatography/electrospray mass spectrometry was then optimized by injections of the synthetic standard. A 6-point standard curve was developed using peak height ratios of standard analyte to internal standard versus mass of dG-C8-ABP standard spiked into a digest of calf thymus DNA (Figure 2). dG-C8-ABP was spiked at 5, 10, 20.1, 34.1, 54.7, and 68.3 femtomoles. Linear regression gave a slope of 0.249 with *r*² value of 0.968. These levels have been determined sufficient for the quantification of dG-C8-ABP in human samples.

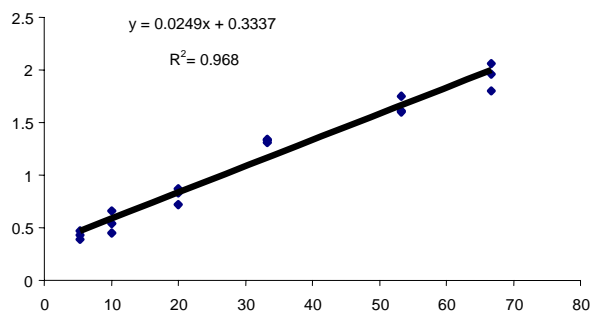


Figure 2: Standard curve of dG-C8-ABP adduct

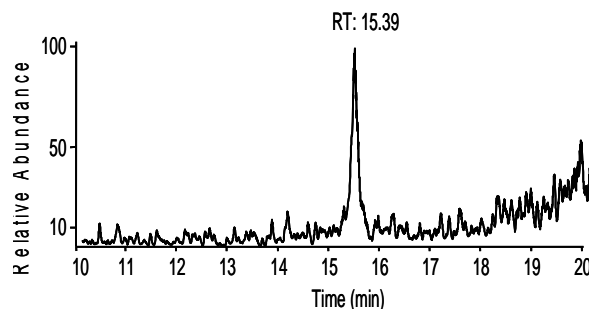


Figure 3: TIC of dG-C8-ABP and dG-C8-ABP-D₉

Total ion current (TIC) for the analysis, shows a single peak at retention time of 15.39 minutes, using selected reaction monitoring, of m/z 435.0 \rightarrow 319.0 and 444.0 \rightarrow 328.1 (Figure 3), indication that both the standard and internal standard have a similar retention time as expected. Thus far we have examined, a 12 sample set of human pancreas DNA, consisting of female and male both smokers and non-smokers, by mass spectrometry for the presence of dG-C8-ABP adducts (Table 1).

Sample ID	Gender	Smoker/ Non-Smoker	Age	IS Area	Analyte Area	Analyte/ IS ratio	fmole of dG-C8-ABP	Adducts/10 ⁸ nucleosides
78	F	NS	17	63972	18670	0.292	< 5	
160	F	NS	20	41342	88712	2.15	72.8	8.10
3	F	NS	42	49010	0	0	<5	
8	M	NS	22	55601	46704	0.840	20.3	2.26
28	M	NS	59	52883	12428	0.235	<5	
41	M	NS	37	55423	764139	13.8	540	60.1
26	F	S	41	50777	0	0	<5	
59	F	S	63	51422	86159	1.68	53.9	6.00
62	F	S	51	1	0	0	no data	
152	M	S	23	53680	18258	0.340	<5	
159	M	S	24	49554	272996	5.51	208	23.1
163	M	S	23	20932	12366	0.591	10.3	1.15

Table I. Quantification of dG-C8-ABP in Human Pancreatic Tissue Samples

The samples were divided according to gender and smoking preference and were placed into four subcategories: female non-smokers, male non-smokers, female smokers, and male smokers. Each of the four subcategories consisted of three samples. Prior to analysis of the human DNA samples, system blanks and procedure blanks were processed and analyzed along with the standard curve used in the quantification of the adducts, and adduct presence was not detected. A second procedure blank was developed in a similar manner as the standard curve with the substitution of the magnesium chloride/TRIS buffer for the synthetic standard; trace amount of adduct in calf thymus DNA was detected. Therefore, the lowest standard peak of the calibration curve was compared with the peak in the blank digest. It was determined that the lowest standard peak, 5-fmole of dG-C8-ABP, was 5.5-fold greater than the amount found in the blank digest, and was thus used as the limit of detection. The presence of dG-C8-ABP adducts was found in 6 of subjects, with no correlation between smokers and non-smokers. The highest adduct content, 60.1 adducts in 10⁸ nucleosides, was found in a male non-smoker. The lowest adduct content, 1.15 adducts in 10⁸ nucleosides, was found in a male smoker.

Conclusion: Adducts were found in six subjects, with no correlation between smokers and non-smokers. The results presented provide evidence to the applicability of LC-MS for the detection and quantification of 4-aminobiphenyl adducts in human tissues. Furthermore, LC-MS may provide a sensitive method to quantitatively screen human subjects for aromatic amines found in cigarette smoke and determine the level of DNA adducts for general risk assessment.