Measurement of Polycyclic Aromatic Hydrocarbon Hemoglobin Adducts by High Resolution Mass Spectrometry

Ragin, A, Crawford, K, Wright A, Grainger G, Patterson, DG Jr.

National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford, NE, Chamblee, Georgia

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment and encompass a class of chemical carcinogens which play a significant role in the onset of a number of human cancers.[1, 2] Many PAHs are produced and released into the environment from incomplete combustion of fossil fuels, industrial production of petroleum products and automobile exhaust.[3] The principal routes of human exposure to PAHs result from cigarette smoke and dietary intake. PAH exposure studies have generally been conducted via analytical methods utilizing HPLC/ fluorescence or GC/MS measurement of hydroxy-PAH metabolites in urine.[4-9] These metabolites have a detection window of approximately 72 hours following exposure. PAH adducts with proteins, however, potentially generate an extended window (120 days) for analytical measurements because they integrate the biological effective dose over the lifetime of the protein. Protein adducts are considered potentially valuable biomarkers for assessment of PAH exposure, with a preponderance of studies using hemoglobin or serum albumin as target macromolecules.

Benzo(a)pyrene (BaP) is one of several PAHs considered among the most carcinogenic, and as a result, is the most extensively studied PAH.[10] We have developed a high resolution gas chromatography mass spectrometry method for measuring isomeric benzo[a]pyrene (BaP) tetrols resulting from hydrolysis of PAH-hemoglobin adducts. Using our method, we applied mass, retention time and fragmentation parameters to the analysis of derivatized benzo[a]pyrene tetrol isomers. We have observed the occurrence and distribution of isomeric BaP tetrols derived from benzo[a]pyrene diolepoxide (BPDE) hemoglobin adducts extracted from the blood of a cohort of smoking and non-smoking human subjects. We are currently developing a quantitative gas chromatography/high resolution mass spectrometry method for assessment of human exposure to PAHs through isotope-dilution measurements of hemoglobin adducts levels.

Materials and Methods

The native benzo(a)pyrene tetrol isomer standards, (+/-)-BaP-r-7,t-8,t-9,c-10-tetrol (BPTI-1), (+/-)-BaP-r-7,t-8,t-9,t-10-tetrol (BPTI-2), (+/-)-BaP-r-7,t-8,c-9,t-10-tetrol (BPTII-1) and (+/-)-BaP-r-7,t-8,c-9,c-10-tetrol (BPTII-2) were obtained from the NCI Chemical Carcinogen Repository, Midwest Research Institute (Kansas City, MO). The BaP tetrols were used without further purification. Solid phase extraction (SPE) cartridges (Sep-Pak 1 g C18) were obtained from Waters (Milford, MA). The derivatizing agent, N–methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) was obtained from Sigma Chemicals (St Louis, MO). Human blood samples were obtained from volunteer donors.

Blood, 10 mL, was drawn from a non-smoking volunteer donor. The red blood cells (RBC) were separated from whole human blood by centrifugation. The red blood cells were washed 3 times with 0.9% (w/v) sodium chloride solution. The RBCs were suspended in phosphate buffered saline (PBS, pH 7.4), then lysed with lysis buffer. The cell lysate was added dropwise to a solution of HCl/acetone while stirring at -20^{o} C to precipitate the hemoglobin (Hb). The precipitated hemoglobin was collected by centrifugation and dried under a constant stream of nitrogen gas at 37^{o}C. After drying, the protein was weighed and fractioned into 200 mg samples in amber glass vials then dissolved in 4 mL of deionized water. The samples were hydrolyzed with 9N HCl for 3 hours at 90^{o} C. The hydrosylates were extracted 3X with isopropyl ether (IPE) or ethyl acetate (EtOAC). The eluents were evaporated to dryness and reconstituted in 20 µL of THF. The samples were subsequently derivatized with N–methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) containing 10% triethylamine (TEA) to convert the PAH tetrols to their corresponding trimethylsilyl (TMS) derivative.

GC-EI-HRMS ANALYSIS. TMS-derivatized tetrols were analyzed on a Thermo-Finnigan MAT-95XL high resolution mass spectrometer (Thermo-Finnigan, San Jose, CA) equipped with an electron impact ionization source and interfaced to an Agilent Technologies 6890 gas chromatograph (GC) system (Agilent Technologies, Palo Alto, CA) equipped with a Finnigan MAT A200S autosampler (Thermo-Finnigan, San Jose, CA). The injector temperature was set at 280 ^{o}C and operated in splitless mode. The oven temperature was held at 100 ºC for 2 minutes, programmed to 310 ºC at 25 ºC for 9 minutes, and then held at 310 ^{o}C for 6 minutes. A J&W DB-5MS 25 m fused silica capillary column (column id 0.25 mm; 25 micron film thickness) was used for analyte separation.

Results and Discussion

The 4 BaP tetrols, (+/-)-BaP-r-7,t-8,t-9,c-10-tetrol (BPTI-1), (+/-)-BaP-r-7,t-8,t-9,t-10-tetrol (BPTI-2), (+/-)-BaP-r-7,t-8,c-9,t-10-tetrol (BPTII-1), (+/-)-BaP-r-7,t-8,c-9,c-10-tetrol (BPTII-2) were characterized by GC-EI-HRMS analysis. The GC-EI-HRMS of TMS derivatized BaP tetrols exhibited a major fragment at 404 (relative intensity 100) with a secondary fragment at m/z of 191. The retention times observed for BPTII_1, BPTI_1, BPTII_2 and BPTII_2 were 12.64, 12.92, 13.50 and 13.98 respectively. GC-EI-HRMS analysis of the sample extracted from a non-smoking donor and purified by liquid-liquid extraction (LLE) with either IPE or EtOAC revealed the presence of the 4 isomeric benzo[a]pyrene tetrols in all samples analyzed (Figures 1 and 2). Representative chromatograms are shown in Figures 3 and 4. The samples and standards (not shown) that were extracted with ethyl acetate exhibited higher area counts for the analytes than the isopropyl ether extracts as observed by GC-EI-HRMS. Purification of PAH tetrols from hemoglobin hydrosylates by LLE of the hemoglobin hydrosylates with ethyl acetate is very promising. The LLE data is more reproducible than what we have observed with solid phase extraction (SPE) of the hemoglobin hydrosylates. With SPE, problems with clogging of the cartridges were frequent and the resulting extracts needed additional clean-up. The isopropyl ether and the ethyl acetate extracts were very clean. However, problems with emulsions were observed when using isopropyl ether as an extraction solvent. We have received ^{13}C labeled standards of the 4 BaP tetrols from Cambridge Isotope Laboratories, Inc. (Andover, MA). We are currently developing a quantitative gas chromatography/high resolution mass spectrometry method for assessment of human exposure to PAHs through isotope-dilution measurements of hemoglobin adducts levels.

Acknowledgements

We would like to acknowledge Susan Welch for her help with GC-EI-HRMS training.

References

1. Conney AH. *Cancer Res* 1982; 42:4875
2. Shields PG, Bowman ED, Harrington AM, Doan VT, Weston A. *Cancer Res* 1993; 53:3486.
3. International Agency for Research on Cancer (IARC). 1984; 34:65.
4. Jongeneelen FJ, Anizon RBM, Henderson PTH. *J. Chromatog* 1987; 413:227.
5. Grimmer G, Dettbarn G, Naaujack KW, Jacob J. *Intern. J. Anal. Cheam* 1991; 43:177.
6. Angerer J, Mannschreck C, Gundel J. *Int. Arch. Occup. Environ. Health* 1997; 69:323.
7. Gundel J, Schaller, KH, Angerer. *Int Arch Occup Environ Health* 2000; 73:271.
8. Heudorf U, Angerer J. *Int Arch Environ Health* 2001: 74:91.
9. Smith CJ, Huang W, Walcott C, Grainger J, Patterson DG Jr. *Bioanal Chem* 2002; 372:216.
10. Conney AH, Chang RL, Jerina DM, Wei SJC. *Drug Metab Rev* 1994; 26:125.

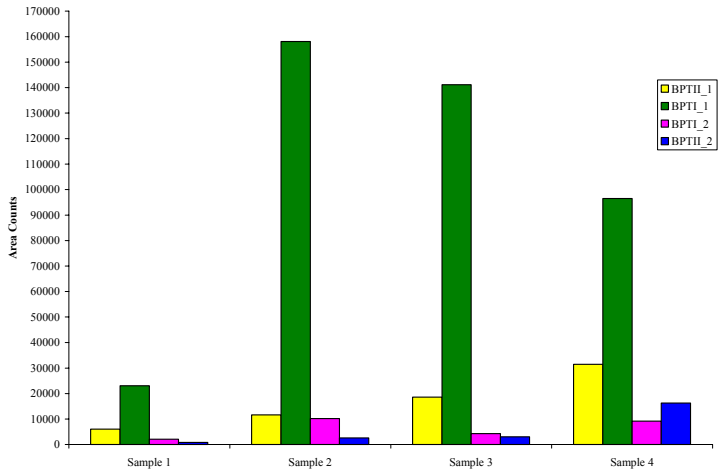

Figure 1. Purification of BaP Tetrols from Hemoglobin Hydrosylates by Liquid-Liquid Extraction with Isopropyl Ether

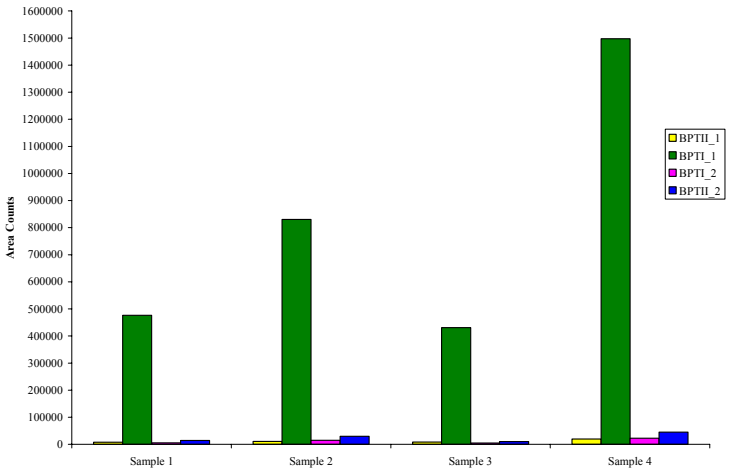

Figure 2. Purification of BaP Tetrols from Hemoglobin Hydrosylates by Liquid-Liquid Extraction with Ethyl Acetate

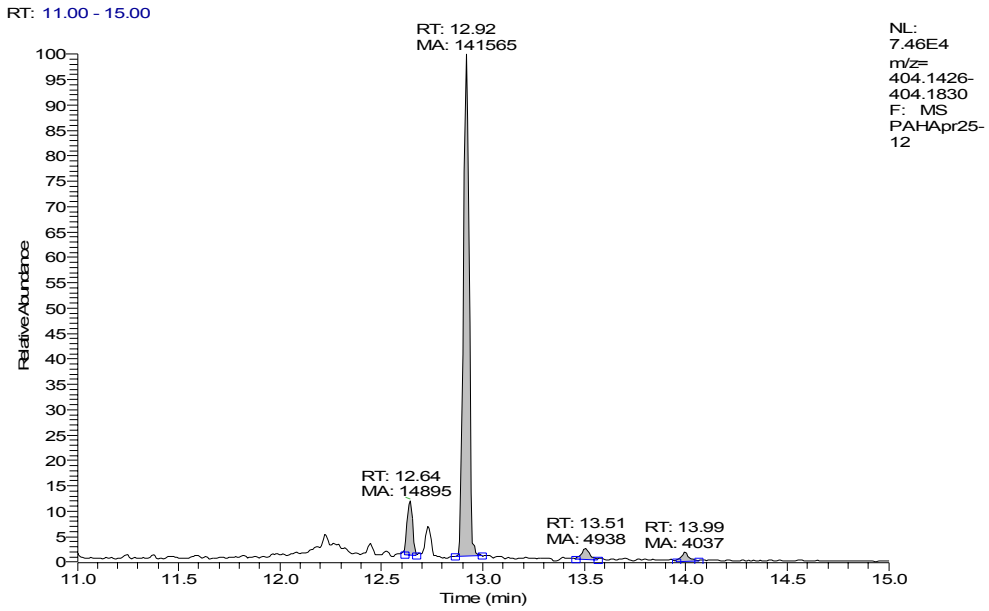

Figure 3. GC-EI-HRMS of Isomeric B[a]P Tetrols Extracted from the Blood of a Non-smoking Donor and Purified by Extraction with Isopropyl Ether

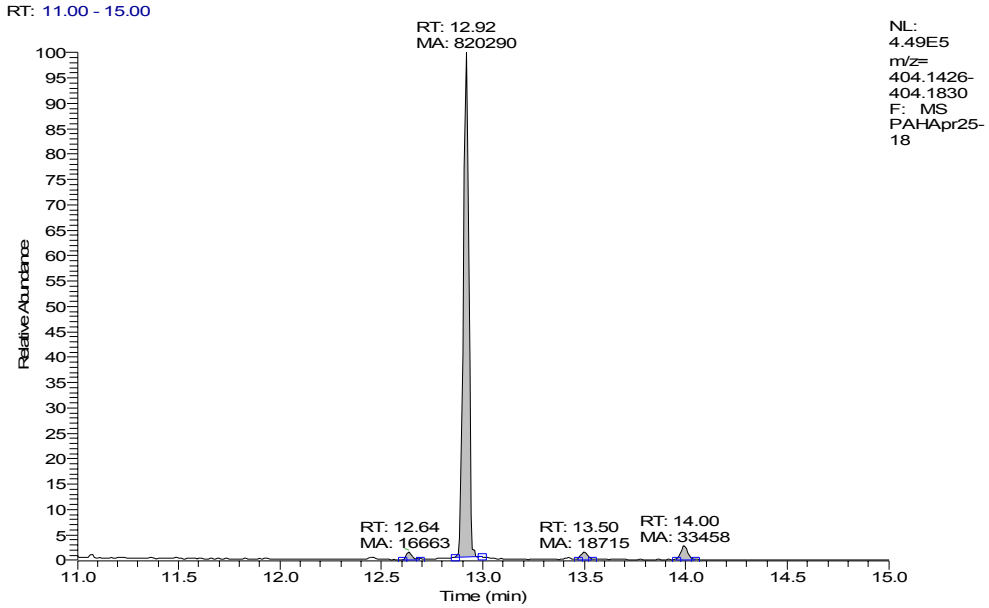

Figure 4. GC-EI-HRMS of Isomeric B[a]P Tetrols Extracted from the Blood of a Non-smoking Donor and Purified by Extraction with Ethyl Acetate