Semi-Automated Method for the Determination of Hydroxylated Metabolites of Polycyclic Aromatic Hydrocarbons in Urine using Gas Chromatography High-Resolution Mass Spectrometry (GC/HR-MS)

Zheng Li[1], Lovisa Romanoff[1], Nasira Hussain[1], Debra Trinidad[1], Donald G Patterson Jr.[1], Andreas Sjodin[1]

[1]National Center for Environmental health (NCEH), Division for Laboratory Sciences (DLS), Centers for Disease Control and Prevention (CDC)

Exposure to polycyclic aromatic hydrocarbons (PAHs) has been linked to a number of adverse health effects in humans including cancer. Hence it is of importance to monitor exposure of these compounds in the general US population and identify specific populations that may be at an increased risk of exposure. PAHs are metabolized in the body to hydroxylated metabolites (OH-PAHs) that are excreted in urine and feces. The quantitative determination of these metabolites in these matrices thus gives information on the subjects' recent exposures to PAHs. OH-PAHs have been included in the National Health and Nutrition Examination Survey (NHANES) in two previous reports. In this abstract an improved method for the determination of OH-PAHs in human urine is presented. This method will be the standard approach to determine OH-PAHs in the NHANES 2003-04 survey as well as other studies involving the measurement of OH-PAHs in human urine at the CDC.

OH-PAHs are conjugated with glucuronic acid or sulfate, to facilitate urinary excretion. Our approach for measuring OH-PAHs in urine includes enzymatic hydrolysis of these conjugates by the addition of sodium acetate buffer (1M; 1mL) with dissolved glucuronidase/arylsulfatase enzyme (10 mg/mL) derived from Helix Promatia. ^{13}C-labeled internal standards (IS; n=13) were also added to the samples before overnight de-conjugation at 37^{o}C. The following day the samples were transferred to a Gilson 215 liquid handler (Middleton, WI) for automated liquid/liquid extraction. The extracts were then fortified with dodecane (5ul), evaporated, reconstituted with toluene and spiked with recovery standard. After transfer to GC-vials, the samples were derivatized to yield the trimethylsiloxane analog. The extracts were finally analyzed using gas chromatography high resolution mass spectrometry (GC/HRMS). A total of 24 OH-PAHs were included in the measurements, including the mono-hydroxylated metabolites of Naphthalene (n=2), Fluorene (n=3), Fluoranthene (n=1), Phenanthrene (n=5), Pyrene (n=1), Benzo(c)phenanthrene (n=3), Benzo(a) anthracene (n=2), Chrysene (n=5), and Benzo(a)pyrene (n=2).