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Thaneeya CHETIYANUKORNKUL[a], Tippawan Prapamontol[b], Akira Toriba[c], Tang Ning[d], Takayuki Kameda[e] and Kazuichi Hayakawa[f]

(a) Environmental Science Program, Faculty of Science, Chiang Mai University, Mueng, Chiang Mai, 50-200, THAILAND
(b) Research Institute for Health Sciences, Chiang Mai University, Mueng, Chiang Mai, 50-200, THAILAND
(c) (d) (e) (f) Graduate School of Natural Science and Technology, Kanazawa University, Kakuma-machi, Kanazawa, Ishikawa, 920-1192, JAPAN

Polycyclic aromatic hydrocarbons (PAHs) are formed and emitted into the environment as a result of incomplete combustion of organic materials from nature and human active processes. Some PAHs are carcinogenic or co-carcinogenic compounds. Biological monitoring of urinary metabolites of PAHs can be used as a feasible way to access the exposure to multiple environmental contaminants such as PAHs [1, 2]. We developed a method for determining monohydroxy polycyclic aromatic hydrocarbons (OHPAHs) having 2-, 3- and 4-rings in human urine by using high performance liquid chromatography with fluorescence detection. Ten OHPAHs, 1- and 2-hydroxynapthalenes (1- and 2-OHNap), 2-hydroxyfluorene (2-OHFle), 1-, 2-, 3-, 4- and 9-hydroxyphenanthrenes (1-, 2-, 3-, 4- and 9-OHPhe), 3-hydroxyfluoranthene (3-OHFrt) and 1-hydroxypyrene (1-OHPyr) were used as multi-biomarkers to estimate the exposure to PAHs. As an application, characteristics of urinary OHPAHs of subjects lived in Chiang Mai Province, Thailand were investigated.

We successfully developed an analytical method for simultaneous determination of 10 kinds of OHPAHs, and use of the deuterated compound as an internal standard allowed them to be accurately quantified. To separate 1-OHPyr and the internal standard (1-OHPyr-d_9), we chose an alkylamide-type reversed phase column. Figure 1 shows typical chromatograms of a standard mixture of ten OHPAHs and a Thai non-smoker taxi driver's urine. The latter chromatogram shows that ten kinds of OHPAHs, 1- and 2-OHNaps, 2-OHFle, 2-, 3- and 4-OHPhes, 1- + 9-OHPhes and 1-OHPyr in human urine were quantitatively separated. 1-OHPyr-d_9 eluted prior to the non-deuterated compound with enough resolution (R_s, 1.45). The limits of detection (S/N=3) were in the range from 2.3 fmol (1-OHPyr) to 2.2 pmol (1-OHNap). Good linearities of the calibration curves were obtained for all OHPAHs ($0.996 \leq r^2 \leq 0.999$). After treatment of urine sample with the SPE cartridges (C_{18} and Silica), the peaks of the ten OHPAHs were free from any interfering peaks, though several interfering peaks were observed around the peaks of OHNaps (Figure 1). The analytical intra-day (n = 5) and inter-day (n = 5) data of the ten urinary OHPAHs are acceptable with variation in the values from 1.6 (3-OHFrt) to 17 (2-OHPhe) % for precision and 92.3 (3-OHFrt) to 119 (3-OHPhe) % for accuracy. These values indicate that 1-OHPyr-d_9 is an excellent internal standard and the proposed method is satisfactory for determining OHPAHs in human urine.

[a] scitchty@chiangmai.ac.th
[b] tippawan@rihes.cmu.ac.th
[c] toriba@p.kanazawa-u.ac.jp
[d] tang@p.kanazawa-u.ac.jp
[e] kameda@p.kanazawa-u.ac.jp
[f] hayakawa@p.kanazawa-u.ac.jp

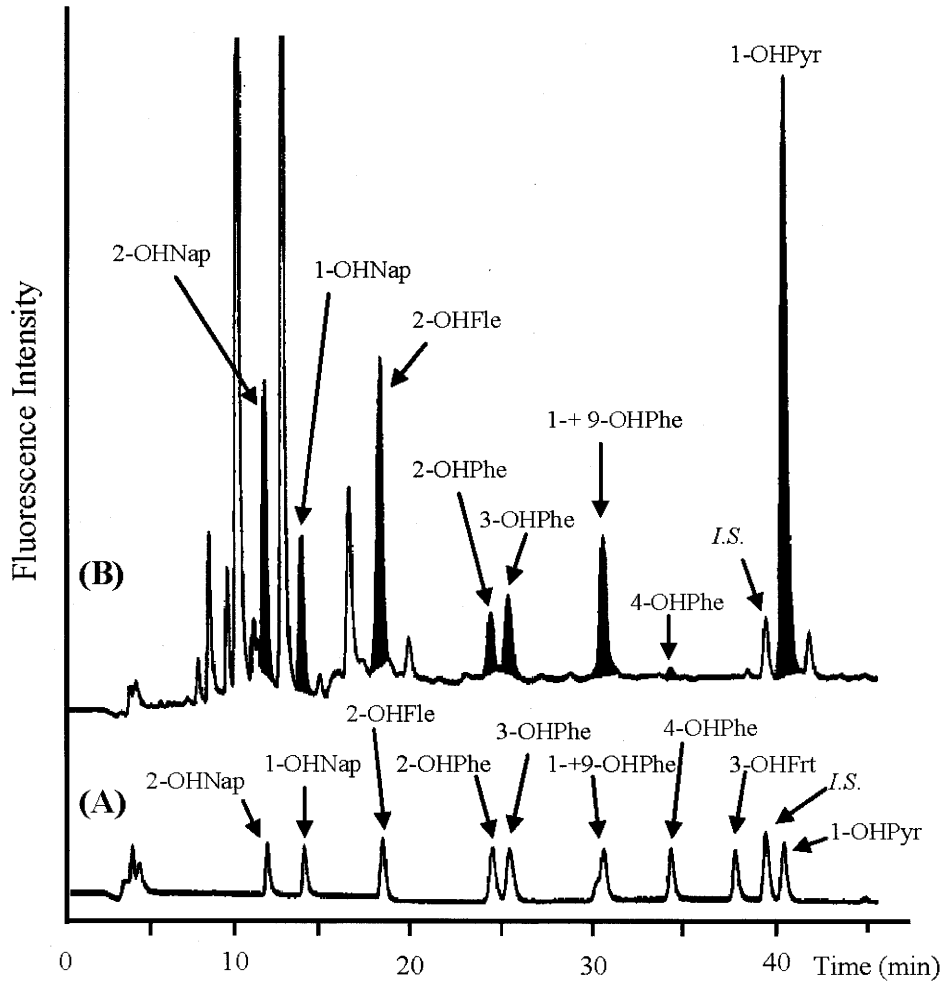

Fig. 1 Representative HPLC chromatograms of a standard sample (A) and a Thai non-smoker urine sample (B).

By using the proposed method, OHPAHs were quantified and compared in urine samples of subjects who lived in Chiang Mai, Thailand. The subjects were divided into three groups including rural villagers, taxi drivers and traffic policemen. The mean concentrations of OHNaps (normalized to the concentration of creatinine) were much higher than those of 2-OHFle, OHPhes and 1-OHPyr in all groups. The concentrations of urinary OHPAHs increased with decreasing ring number. 3-OHFrt was not detected in any of the urine samples tested in this study. The most interesting result was that the concentrations of all detected metabolites, except for 1-OHNap, of rural villagers were significantly higher than those of the other two groups. In contrast, no significant difference in any of the metabolites was found between the taxi drivers and the traffic policemen. The high urinary levels of OHPAHs of rural villagers are thought to be mainly due to atmospheric PAHs produced by open-burning for the agricultural purpose and by burning of biomass (wood and charcoal) for cooking and heating. Thus, the urinary profile of OHPAHs can serve as multiple biomarkers which reflect the exposure to PAHs from the environment and human activities.

References

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