Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 805 (2004) 187–193
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Determination of 1-hydroxypyrene in children urine using column-switching liquid chromatography and fluorescence detection

Ching-Tang Kuo [a,b,*], Hong-Wen Chen [c,d], Jiann-Lin Chen [a]

[a] Institute of Environmental Health, China Medical University, 91 Hsueh-Shih Road, Taichung 404, Taiwan, ROC
[b] School of Risk Management, China Medical University, 91 Hsueh-Shih Road, Taichung 404, Taiwan, ROC
[c] Department of Environmental Engineering and Health, Yuanpei University of Science and Technology, 306 Yuanpei Street, Hsinchu 300, Taiwan, ROC
[d] Institute of Environmental Engineering, National Chiao Tung Unversity, 75 Po-Ai Street, Hsinchu 300, Taiwan, ROC

Received 17 February 2003; received in revised form 19 November 2003; accepted 16 December 2003

Available online 24 April 2004

Abstract

This study developed an acid hydrolysis method instead of using enzyme extraction, equipped with column-switching system for the pretreatment of samples, in the determination of 1-hydroxypyrene in the urine from children and pyrene in airborne particulates. We collected both types of samples from areas near a petrochemical industry and rural areas as reference. Samples were first treated with acid hydrolysis and followed by solvent extraction prior to being injected into the separation system for the determination with high performance liquid chromatography and fluorescence. A column-switching system was on-line with a C_{18} separation column to remove matrix interference and obtain a stable baseline of the chromatogram. The eluent used to separate the 1-hydroxypyrene was 60% (v/v) aqueous acetonitrile solution. A fluorescence detector was used to monitor 1-hydroxypyrene at $\lambda_{ex} = 348$ nm and $\lambda_{em} = 388$ nm, and pyrene at $\lambda_{ex} = 331$ nm and $\lambda_{em} = 390$ nm. Both calibration graphs were linear with very good correlation coefficients ($r > 0.999$) and the detection limits were ca. 2 pg (5 ng/l). Results showed that there was a significant association between 1-hydroxypyrene levels in urine specimens and pyrene levels in airborne particulate samples ($r = 0.68$, $P < 0.05$). The average levels of pyrene in the particulates (0.18 versus 0.09 ng/m^3) and of 1-hydroxypyrene in urine specimens (155.9 versus 110.2 ng/g creatinine) were higher for the petrochemical area than for the rural area. This method is stable and sensitive for measuring polycyclic aromatic hydrocarbons in environmental samples.
© 2003 Elsevier B.V. All rights reserved.

Keyword: 1-Hydroxypyrene

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of well-known environmental carcinogens [1]. The International Agency of Research on Cancer has listed 11 PAHs as carcinogenic based on animal studies [2]. The most prevalent source of PAHs is from the combustion of organic materials [3]. Humans may expose to these chemicals from a variety of sources, including polluted air, polluted water, smoking, broiled foods and occupational setting such as coke-oven industry [4–8]. As a typical PAH, pyrene is metabolized extensively into 1-hydroxypyrene and then excreted via feces and urine [9–12]. Urinary 1-hydroxypyrene is thus used as a biological marker of PAHs exposure [13,14].

Several analytical techniques have been adapted for the determination of 1-hydroxypyrene in urine [15–22], employing enzyme hydrolysis of the conjugates, followed by solid-phase extraction prior to chromatographic determination [15–18]. These methods are effective in the measurement of urinary 1-hydroxypyrene, but the procedures of sample treatment are complicated. It still takes 28 min to complete one experiment even a tailor-mode equipped with copper phthalocyanine-modified porous silica pre-column, and dichloromethane/ethylacetate silica gel column is used for the clean-up process [19–21]. It is also an expensive and time-consuming process.

In the present study, we developed a column-switching liquid chromatographic technique to determine urinary 1-hydroxypyrene after acid hydrolysis and solvent extraction

* Corresponding author. Tel.: +886-4-22070429; fax: +886-4-22070429.
E-mail address: ctkuo@mail.cmu.edu.tw (C.-T. Kuo).

1570-0232/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2003.12.012

for the sample. In addition, we applied this method for the routine biological monitoring of urinary 1-hydroxypyrene for two groups of children, by comparing children living in an area with petrochemical industry with children living in a rural area. Pyrene in air particulates was also measured for these two areas.

2. Experimental materials and methods

2.1. Apparatus

Fig. 1 shows the schematic diagram of the instrument set-up. Using a fluorescence spectrophotometer (RF-5000, Kyoto, Japan) as the detector, the high performance liquid chromatography system was equipped with two HPLC gradient pumps, Model LC-10AD and SCL-10A (Shimadzu, Kyoto, Japan), an automatic sample injector with six-ports switching valve (FCV-2AH, Shimadzu, Kyoto, Japan), a pre-column (Kaseisorb LC ODS-60-5, 50 mm × 4.6 mm i.d., Tokyo, Japan) and a reversed-phased analytical column (Kaseisorb LC ODS-60-5, 250 mm × 4.6 mm i.d., Tokyo, Japan). The temperature of the analytical column was maintained at 30 °C with thermostat oven (Shimadzu model CTO-6A, Kyoto, Japan) at a flow-rate of 1.0 ml/min. The analytical data were recorded on a microprocessor integrator using Chem Win 1.0 system (Taipei, Taiwan). Creatinine in urine was determined photometrically according the Honglan methods [23]. A high volume sampler (PM_{10}, Kimoto Denshi, Osaka, Japan) was used to collect air particulates of <10 μm in diameter (PM_{10}).

The analysis of low chemical levels in urine requires the use of a very sensitive and accurate method. In order to improve the sensitivity, 1-hydroxypyrene was pre-concentrated with a reversed-phase pre-column. The impurity of urinary metabolites was spilt and cleaned up using the paired HPLC pumps with programmable switching-column and valves. The concentrated urinary 1-hydroxypyrene was detected using a fluorescence spectrophotometer. Both the excitation and emission slits were set to 10 nm. The detection wavelengths were changed during each run to measure separate metabolites with optimal sensitivity and specificity. The

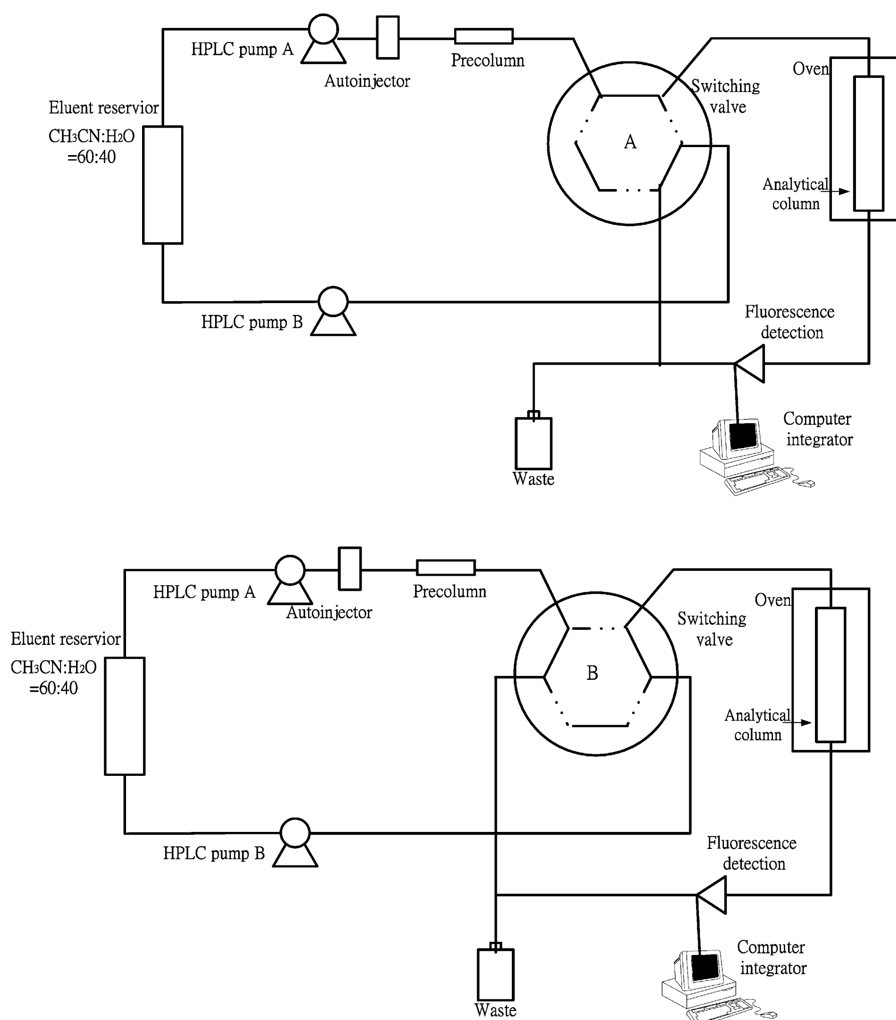

Fig. 1. Scheme of instrumental arrangement and procedure of column-switching mechanism described in Section 3.1.

fluorescence wavelengths were set at $\lambda_{ex} = 348$ nm and $\lambda_{em} = 388$ nm for the determinations of urinary 1-hydroxypyrene at $\lambda_{ex} = 331$ nm and $\lambda_{em} = 390$ nm for pyrene.

2.2. Chemicals and reagents

Chemicals used in this study were all of ACS grade. 1-Hydroxypyrene was obtained from Aldrich (Milwaukee, WI, USA). Pyrene and acetic acid were purchased from TCI (Tokyo, Japan). Hydrochloric acid, sodium hydroxide, dimethyl sulfoxide, and sodium hydrogen sulfoxide were obtained from E. Merck (Darmstadt, Germany). The HPLC grade solvents, benzene, ethanol, acetonitrile, 2-proanol, methanol, and diethyl ether were obtained from Fisher (Springfield, MO, USA). Creatinine and β-glucuronidase/sulfatase (97,000 U) were obtained from Sigma (England). Using the Millipore 60 system (Bedford, MA, USA), the distilled de-ionized water (conductivity: >18 M Ω/cm) was used for all aqueous solutions. 1-Hydroxypyrene stock solution was prepared by dissolving 5 mg chemical in 100 ml 2-propanol. The pyrene stock solution contained 50 mg chemical in 100 ml dimethyl sulfoxide (DMSO). All solutions were stored in brown bottles and kept in a −20 °C refrigerator. The mobile phase for 1-hydroxypyrene contained 60% aqueous acetonitrile solution, and the eluent for the pyrene analysis consisted of 85%. All eluents were degassed using helium for 30 min.

2.3. Study subjects, sample collection and preparation

2.3.1. Pyrene sampling

Study subjects were children from two elementary schools. At Hoping elementary school located near the petrochemical district in Kaoshiung, Taiwan, was designated as study area. Nanto elementary school in the rural area of Nanto County was designated as reference area. Two types of sample were collected. One was airborne particulate sample for the measure of pyrene and the other one was children's urine sample for the measure of 1-hydroxypyrene. Pyrene samples were collected using high volume sampling technique, 10 m above the ground at schools in the study areas. The pyrene particulates were captured on glass fiber filter (10 in. × 8 in., Gelman Science, Michgan, MI, USA) for 1 week. The samplers were run for 24 h continuously at a flow rate of approximately 1.1 m^3/min and were calibrated about everyday. All filter samples were stored in dark at −20 °C until analysis.

2.3.2. Urine sampling

Since children are sensitive to PAHs exposure, the children were randomly chosen from the selected schools. Spot urine samples were collected from 18 boys and 18 girls (mean age: 11 years) at 3:00–4:30 p.m. at school. Each participant was asked to answer a questionnaire for information including age, transportation status, dwelling conditions, and other potential sources of PAHs exposure. An aliquot was taken from each fresh urine sample for creatinine determination and the adjustment of 1-hydroxypyrene concentration. The urine samples were portioned in 50 ml tubes and kept at −20 °C until analysis.

2.4. Sample pretreatment

2.4.1. Pyrene extraction

Pyrene in the particulate was extracted in an ultrasonic bath. The sample filter was cut by stainless cutter and placed in a centrifuge tube (Pyrex, 10 ml). Each filter sample was rinsed with 2 ml of ethanol and immersed in 6 ml of benzene. The tube was placed in an ultrasonic ice-bath for 10 min, and then centrifuged for 5 min (3000 rpm). A 3 ml of the supernatant was liquid–liquid extracted with 4 ml of 5% sodium hydroxide. The organic phase was injected into the analytical system. As in our previous study [24], pyrene in the particulate was extracted ultrasonically with benzene. However, benzene is a toxic chemical and classified as the human carcinogens in IARC Monographs. We use lower toxicity acetonitrile to replace benzene. The average recovery of airborne pyrene reached 98%.

2.4.2. Acid hydrolysis and urinary pretreatment

Since acid hydrolysis is more feasible than traditional enzymatic hydrolysis for urinary 1-hydroxypyene, a 10 ml aliquot of the urine sample was hydrolyzed by adding 2 mg of sodium chloride, 2 ml of hydrochloric acid, and heated for 1 h (60 °C). After heating, the hydrolyzed sample was extracted with 20 ml diethyl ether in a shaker. The organic phase was evaporated to dryness at 37 °C with a gentle stream of nitrogen gas. The residue was reconstituted with 1 ml of 2-propanol and injected into LC-FLD for determination.

In order to select an appropriate solvent for the extraction of 1-hydroxypyrene, four solvents (toluene, benzene, dichloromethane, and diethyl ether) were compared. Blank urinary samples were spiked with 1-hydroxypyrene, extracted and pretreated as the procedure described previously, and then analyzed by the proposed column-switching HPLC/FLD. The average values of extraction efficiency ($n = 5$ each) were 48.56% with 8.23% R.S.D. for toluene, 66.24% with 0.35% R.S.D. for benzene, 64.38% with 1.52% R.S.D. for dichloromethane, and 81.62% with 2.73% R.S.D. for diethyl ether. Thus, diethyl ether was used to extract 1-hydroxypyrene through this study (Table 1).

For obtaining an optimal efficiency of acid hydrolysis, parameters that affected the acid hydrolysis such as temperature and time were studied. Fig. 2 shows the effects of the temperature on the hydrolysis efficiency during 1 h reaction. The fluorescence response increased as the temperature increased and reached an optimal value at 60 °C. This phenomenon depicts the transformation of 1-hydroxypyrene into other derivatives at the temperature >60 °C during acid hydrolysis. The formation of other products shall cause some interference and reduce the sensitive of 1-hydroxypyrene

Table 1
Comparison of extraction efficiency for 1-hydroxypyrene in different solvents

Solvent	Extraction efficiency	
	Recovery (%)	R.S.D. (%)
Toluene	48.56	8.23
Benzene	66.24	2.35
Dichloromethane	64.38	2.52
Diethyl ether	85.62	2.73

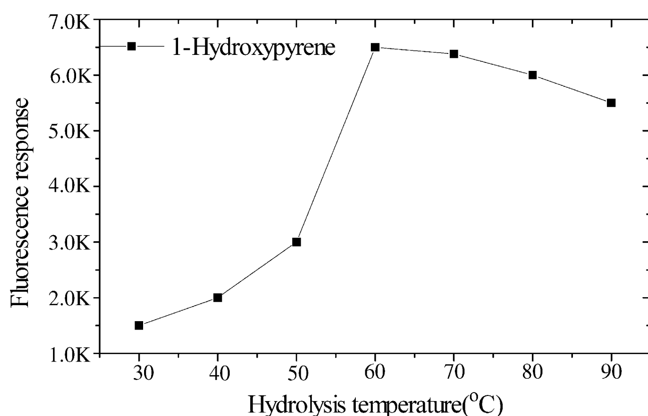

Fig. 2. Effect of the hydrolysis temperature on detection of response.

analysis at high temperature. Fig. 3 shows the kinetic curve of acid hydrolysis of conjugated 1-hydroxypyrene at 60 °C by the hydrolysis time for four urine samples. The fluorescence responses to 1-hydroxypyrene increased with reaction time until 60 min.

2.5. Calibration and recovery tests

Urine samples from children not-exposed to PAHs in reference area (blank urine) were spiked with three levels (6.87, 13.74 and 27.48 ng/ml) of 1-hydroxypyrene standard for the recovery study, allowing the calibration range

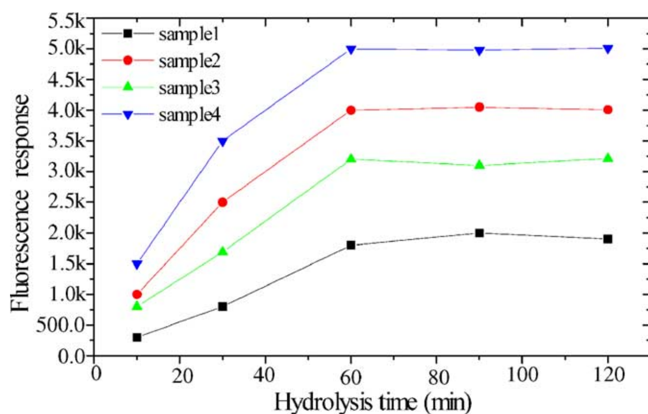

Fig. 3. The kinetic curve of acid hydrolysis obtained from four urine samples.

Table 2
Column-switching analytical procedure

Function	Separation	Analysis	Stable	
Valve position	A	B	A	B
Pump flow (ml/min)				
Pump A	1	2	1	1
Pump B	1	1	1	1
Sample injection (min)				
	0	3.8	12	14

Position A is normal mode. Position B is switched. Mobile phase is $CH_3CN:H_2O = 6:4$ (v/v). Pump A and pump B are used to carry out the eluent of 60% acetonitrile.

extended from 0.07 to 51.00 ng/ml. The standard solution contained 1.02 μg/ml of 1-hydroxypyrene in 2-proponal. Moreover, aliquots (30 μl) of pyrene standard solution was added to sample filter, at seven levels ranged from 13.92 to 1127.78 ng/ml. These calibration samples were treated and analyzed using above described procedure. All spike tests were measured in triplicate. Recovery yields were calculated based on the regression line of standard solutions in the same concentration range.

2.6. Statistical analysis

In addition to measure the efficacy of the sample treatment and assay, data analyses measured Pearson's correlation coefficients (r) between the 1-hydroxypyrene levels in urine and pyrene levels in air particulates, and compared the average levels 1-hydroxypyrene and pyrene in samples obtained from petrochemical area with that obtained from the rural area. Statistical analyses were conducted in SPSS/PC$^+$ (SPSS, Inc., Chicago, IL). All tests were regarded as significant when $P < 0.05$.

3. Results and discussion

3.1. Optimization program of column-switching and clean-up

Using the conventional procedure, the analysis of extracted samples is affected by outside interferences and poor baseline stability due to the complexity of sample matrix. In order to improve the quantitative determination, a "pre-column" was served as a heart-cutting technique to clean-up the interferences in urinary sample prior to the analytical column. The column-switching scheme is illustrated in Fig. 1. The switching valve and HPLC-pumps controlled interference removal. Table 2 shows the timetable to switch column and the flow rates of pumps.

As shown in Fig. 1 and Table 2, the sample aliquot was injected at A status and eluted through the pre-column first with eluent solution at the beginning. The switching valve was switched to B status at 3.8 min and remained until

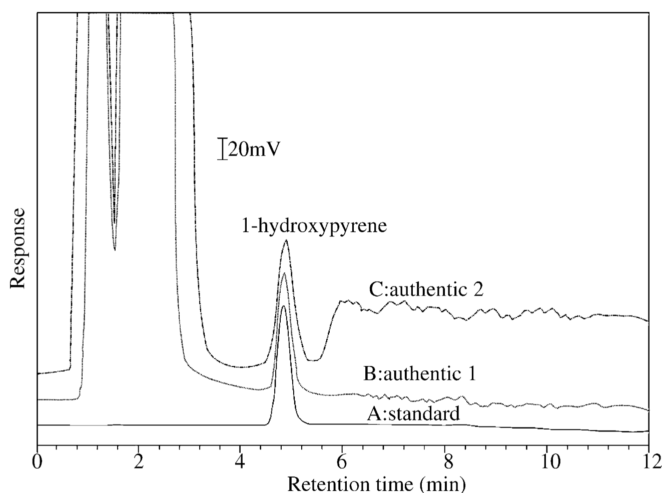

Fig. 4. LC-FLD chromatograms of standard sample and authentic samples. (A) Standard sample (0.63 ng/ml). (B) Authentic sample 1: the chromatogram of using column-switching method (0.50 ng/ml). (C) Authentic sample 2: the chromatogram of off-line purification analysis method (0.50 ng/ml). Response is fluorescence response intensity.

12 min for heart-cut and clean-up. The chromatographic system ran at in A status during the period of 12–14 min and return to B status after 14 min. The gradient flow of acetonitrile for pump A was kept at 1.0 ml/min for 3.8 min, linearly increased to 2.0 ml/min over 8 min and then back to 1.0 ml/min until the completion of analysis. By the completion of each analysis, the pre-column was cleaned up while the new sample was injected into the sample loop at status A. The flow-rate of pump B was kept at 1.0 ml/min during the running procedure.

A pre-column contained C_{18} stationary phase (50 mm × 4.6 mm) with 60% (v/v) aqueous acetonitrile as an eluent provided the best efficacy for clean-up and separation among several tested conditions. This procedure demonstrated an optimal condition in interference removal. Under this condition, the chromatogram of the authentic sample was clear with no residue present, with a good resolution, and with a steady baseline similar to that obtained from the analysis of standard sample. The analytical chromatogram for the sample by separate cut purification displayed the fluctuated baseline with some interferences by other species (Fig. 4). We consider this on-line column-switching method for the analysis of urinary 1-hydroxypyrene a time saving, sensitive and reliable process.

3.2. *Hydrolysis of conjugates and extraction efficiency*

This study compared the efficiency of acid hydrolysis at the optimized conditions with that of enzymatic hydrolysis, using 30 μl of standard materials of 6.87, 13.74, and 27.48 ng/ml, spiked into blank urine specimen. Table 3 shows the average recovery rates for both hydrolysis methods. No significant differences of hydrolysis efficiency were found between these two methods. Nevertheless, it took

Table 3
Comparison of hydrolysis efficiency for 1-hydroxypyrene in different methods

Concentration (ng)	Acid hydrolysis		Enzymatic hydrolysis	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
6.78	86	5.5	88	9.5
13.74	88	2.9	87	0.8
27.48	85	1.8	86	2.0

R.S.D.: relative standard deviation.

a longer incubation time for the enzymatic hydrolysis to ensure the completeness of the reaction.

3.3. *Chromatographic separation and fluorescence detection*

3.3.1. *Optimization of eluent condition and identification of analytes*

In the optimization tests for the best aqueous solution, we found that 60% acetonitrile offered adequate separation of 1-hydroxypyrene from other species co-injected. The optimal eluent composition for pyrene analysis consisted of 85% aqueous acetonitrile solution with isocratic elution. Figs. 4 and 5 show the chromatograms of standard samples and authentic samples for 1-hydroxypyrene and pyrene, respectively, at the optimal separation and detection conditions. The retention behaviors of samples were comparable with that of standards.

3.4. *Detection limit, precision, and linearity*

Calibration plots were constructed for 1-hydroxypyrene and pyrene over the concentration is the ranges of

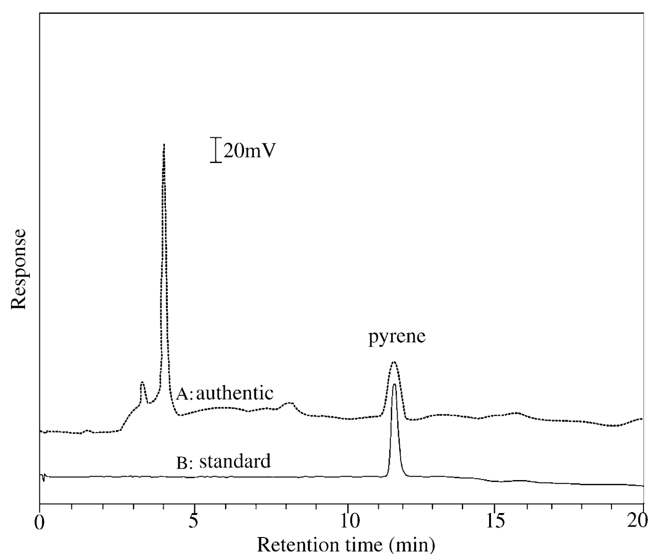

Fig. 5. LC-FLD chromatograms of standard sample and authentic sample. (A) Standard sample (41.78 ng/ml). (B) Authentic sample: the chromatogram of airborne pyrene (21.00 ng/ml). Response is fluorescence response intensity.

Table 4
Reproducibility of peak area and retention time in analytes

Compounds	Calibration range (ng/ml)	Linearity	LOD (pg)	Peak area (R.S.D., %)	Retention time (R.S.D., %)
1-Hydroxypyrene	0.07–51.0	0.999	2	2.40	0.32
Pyrene	13.9–1127.7	0.999	1	0.76	0.29

Table 5
Methodological comparison in1-hydroxypyrene determination among latest studies

Method	Present study	[25]	[26]	[27]
Extraction method	Acid hydrolysis	β-Glucuronidase hydrolysis	β-Glucuronidase hydrolysis	On-line extraction
Extraction recovery (%)	85–90	93	Unknown	99
Recovery (R.S.D., %)	1.8–5.5	8	Unknown	2
Extraction time (min)	60	960	960	20–30
Column-switching use	1	0	0	3
Retention time	4.8	18.5	40	20
LOD (ng/l)	5	57	32	20
Internal standards	None	None	None	None
Resolution	Stable	Unstable	Unstable	Unstable

R.S.D.: relative standard deviation; LOD: limit of detection.

0.05–51 ng/ml and 14–1128 ng/ml, respectively. Least square linear regression analysis was used to estimate (a) slope and (b) intercept of calibration curve $y = ax + b$, where y is the ratio between the peak area of analyte and that of internal standard, and x the concentration of the analyte (ng/ml). The LOD of assay, for pyrene and 1-hydroxypyrene, is calculated according to the expression: $\mathrm{LOD} = 3(\mathrm{SE}_b + b)/a$, where SE_b is the standard error of the intercept, b the intercept and a the slope. The calibration equations were $y = -4.8 + 17.42x$ for 1-hydroxypyrene and $y = 13.50 + 5.14x$ for pyrene. The linear correlation coefficients (r) of both species were above 0.999. The overall repeatability of the assay was estimated using relative standard deviation (R.S.D., %) and calculated by repeating for 10 samples in the analysis for each qualities control sample. This resulted R.S.D. in the range of 0.29–2.40% for peak area and retention time (Table 4). The detection limit was estimated 2 pg for 1-hydroxypyrene in 20 μl injected volume, and 1 pg for pyrene in 10 μl of injected volume. These detection limits were lower than that from the authentic samples of urine and air. But, Table 4 shows that the reproducibility of peak area and retention time is 10 times of analysis. The deviations obtained in this process both for pyrene and 1-hydroxypyrene were <3%.

3.5. Comparison in 1-hydroxypyrene determinations among studies

The determination of urinary 1-hydroxypyrene had been reported in several papers. Table 5 [25–27] compared the present study with other three studies. The proposed method is the best on LOD, extraction time and retention time for the determination of urinary 1-hydroxypyrene. The recovery rates of acid hydrolysis extraction ranged 85–90%, near the results by other methods such as β-glucuronidase enzymatic hydrolysis and on-line extraction. For column-switching technique, Simon et al. [27] have developed a three-valves technique with good accuracy and precision. But, the technique is complex and inconvenient. The present proposed column-switching technique used only one switch-valve was simple, accurate, and practical, and had the accuracy and precision similar to Simon method.

Table 6
Comparison in urinary 1-hydroxypyrene level for children and particulate pyrene concentration between petroleum exposure and reference area

Compound	Exposure area ($n = 36$)		Reference area ($n = 36$)		P-value
	Average	Range	Average	Range	
1-Hydroxypyrene (ng/g creatinine)	155.9	15.4–448.7	110.2	16.9–241.4	<0.05
Pyrene (ng/m^3)	0.18	0.04–0.34	0.09	0.01–0.21	<0.05

Exposure group consisted of the children near petrochemicals in Kaoshiung city, and the reference group the children at a rural area, Nanto.

3.6. Environmental and biological monitoring of polycyclic aromatic hydrocarbons

The average level of pyrene in airborne PM_{10} was substantially higher in samples obtained from the studied petrochemical industry area than that from the rural area (0.18 versus 0.09 ng/m^3, $P < 0.05$) (Table 6). The average level of urinary 1-hydroxypyrene for children living near the petrochemical area was significantly higher than that living in the rural area (155.9 versus 110.2 ng/g creatinine, $P < 0.05$). We consider that the level of urinary 1-hydroxypyrene is associated with the emission of pyrene in solid particulates from the petrochemical plant in Kaoshiung. We found a significant association between urinary 1-hydroxypyrene concentration in children and airborne pyrene ($r = 0.68$, $P < 0.05$). Our results offer a database on the potential risk of environmental exposure to pyrene for children.

4. Conclusion

We have developed an effective extraction process, for the determination of 1-hydroxypyrene in the urinary sample by liquid chromatography, using a switchable clean-up pre-column prior to transiting the analyte to the analytical column. The time programming heart-cut technique is effective to clean-up the complexity from urine matrix. The detection limit of 1-hydroxypyrene is 2 pg (5 ng/l). Recovery yield from acid hydrolysis is above 85%. This study also found that the concentration of urinary 1-hydroxypyrene for children has a strong association with the concentration of airborne pyrene. The proposed system is practicable and reliable, and particularly attractive for routine analysis. This method is sensitive enough to determine the environmental exposure to the low level of polycyclic aromatic hydrocarbons.

Acknowledgements

This work was supported by Taiwan National Science Council Grant NSC 90-2113-M-039-001 and China Medical University Grant CMC91-RM-01.

References

[1] M.N. Kayali, S.R. Barroso, J. Liq. Chromatogr. 18 (1995) 1617.
[2] Internal Agency for Research on Cancer (IARC), Polycyclic Aromatic Compounds. Part 1. Chemical, Environmental and Experimental Data, vol. 38, 1986.
[3] J.P. Buchet, M. Ferreria, J.B. Burrion, T. Leroy, M.K. Volders, P.V. Hummelen, J. Jacques, L. Cupers, J.P. Delavignette, R. Lauwery, Am. J. Ind. Med. 27 (1995) 523.
[4] W. Lijinsky, P. Shubik, Science 145 (1964) 53.
[5] P.J. Lioy, J.M. Waldman, A. Greenberg, R. Harkov, C. Pietarinen, Arch. Environ. Health 43 (1988) 304.
[6] P. Strickland, D. Kang, Toxicol. Lett. 108 (1999) 191.
[7] W.P. Tools, P.B. Show, L.K. Lowry, B.A. Mackenzie, J.F. Deng, H.L. Markel, Appl. Occup. Environ. Hyg. 5 (1990) 303.
[8] A. Morgante, Riv. Merceol. 27 (1988) 83.
[9] E.D. Rahman, J.A. Barrowman, Can. J. Physiol. Pharmacol. 64 (1986) 1214.
[10] C. Roggi, C. Minoia, G.F. Sciarra, P. Apostoli, L. Maccarini, S. Maganaghi, A. Cenni, A. Fonte, G.F. Nidasio, G. Micoli, Sci. Total. Environ. 199 (1997) 247.
[11] D.H. Philips, Mut. Res. 443 (1999) 139.
[12] F.J. Jongeneelen, R.B.M. Anzion, P.T. Henderson, J. Chromatogr. 413 (1987) 227.
[13] F.J. Jongenelen, R.B.M. Anzion, P.T.J. Scheepers, R.P. Bos, P.T. Henderson, E.H. Nijnhus, S.J. Veenstra, R.M.E. Brouns, Ann. Occup. Hyg. 32 (1988) 35.
[14] B.E. Moen, R. Nilsson, R. Nordlineder, K. Bleie, A.H. Skorve, Occup. Environ. Med. 53 (1996) 692.
[15] F.J. Jongeneelen, R.B.M. Anzion, P.T. Henderson, J. Chromatogr. B 665 (1987) 227.
[16] A.M. Hansen, O.M. Poulsen, J.M. Christensen, S.H. Hansen, J. Anal. Toxicol. 17 (1993) 38.
[17] M. Bouchard, C. Dodd, C. Viau, J. Anal. Toxicol. 18 (1994) 261.
[18] R.S. Whiton, C.L. Witherspoon, T.J. Buckley, J. Chromatogr. B 665 (1995) 390.
[19] K.S. Boss, J. Lintelmann, A. Kettrup, J. Chromatogr. 600 (1990) 189.
[20] J. Lintleman, C. Hellemann, A. Kettrup, J. Chromatogr. B 660 (1994) 67.
[21] St. Ferrari, F. Mandel, J.D. Berset, Chemosphere 47 (2002) 173.
[22] T. Chetiyanukornkul, A. Toriba, R. Kizu, T. Makino, H. Nakazawa, K. Hayakawa, J. Chromatogr. A 961 (2002) 107.
[23] S. Honglan, M. Yongqin, M. Yinfa, Anal. Chim. Acta 312 (1995) 79.
[24] C.T. Kuo, H.W. Chen, J. Chromatogr. A 897 (2000) 393.
[25] J. Hollender, B. Koch, W. Dott, J. Chromatogr. B 739 (2000) 225.
[26] J. Gündel, C. Mannschereck, K. Büttner, U. Ewers, J. Angerer, Arch. Environ. Contam. Toxicol. 31 (1996) 585.
[27] P. Simon, Y. Morele, P. Delsaut, T. Nicot, J. Chromatogr. B 732 (1999) 91.