Chiang Mai J. Sci. 2017; 44(4) : 1441-1452
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

A Simple High-performance Liquid Chromatography Coupled to Fluorescence Detection Method using Column-switching Technique for Measuring Urinary 1-hydroxypyrene from Environmental Exposure

Kunrunya Sutan*, Warangakana Naksen* and Tippawan Prapamontol+
Research Institute for Health Sciences (RIHES), Chiang Mai University, Chiang Mai 50200, Thailand.
* These authors contributed equally to this work.
+ Author for correspondence; e-mail: tippawan.prapamontol@cmu.ac.th

Received: 19 December 2016
Accepted: 21 February 2017

ABSTRACT

A method for determination of urinary 1-hydroxypyrene (1-OHP) was developed using HPLC-FLD with a column-switching system. 1-OHP in urine samples was extracted by β-glucuronidase hydrolysis, followed by C_{18} SPE with optimized conditions. LOD and LOQ were 0.01 and 0.03 ng/mL, respectively; this was sensitive enough to determine urinary 1-OHP in low-dose exposure. The precision and accuracy of four concentrations (0.06, 2.0, 8.0 and 25 ng/mL) were tested. The intra- and inter-batch precisions were between 1.82-4.22 and 5.01-15.8 %RSD, respectively, while accuracies were between 92.8-117% recoveries, which were in the acceptable range. This method was applied in 130 school children living in a seasonal forest-fire burning area in Northern Thailand. Urinary 1-OHP was detected in all urine samples at concentrations of 1.35±2.26 μmol/mol creatinine, and a range of 0.03-16.0 μmol/mol creatinine. The developed method offers a potential application to determine urinary 1-OHP levels from environmentally low exposure.

Keywords: 1-hydroxypyrene, HPLC-FLD, column-switching, environmental exposure

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous carcinogenic environmental pollutants derived from the incomplete combustion or pyrolysis of organic materials [1]. Measuring various urinary hydroxylated metabolites has been reported as a biological indicator of exposure to PAHs [2]. 1-hydroxypyrene (1-OHP), metabolite of the non-carcinogen pyrene, is the most common biomarker of exposure to total PAHs in environmental and occupational health studies [3, 4]. Urinary 1-OHP has been associated with many health effects, such as alteration of lung function [5], cardiac autonomic function[6] and thyroid function [7], lipid damage, head and neck, and lung cancer [8, 9] in adults and attention/deficit hyperactivity disorder [10] and cognitive dysfunction [11] in children.

Many chromatographic methods have been published for determining 1-OHP in human urine. High-performance liquid

chromatography (HPLC) coupled to fluorescence detection (FLD) [12-14], tandem mass spectrometry (MS-MS) [15-16] have been used for determination of this compound. Gas chromatography (GC) coupled to MS [17] and MS/MS [18] also have been used to detect urinary 1-OHP in some studies. GC-MS based methods for the determination of hydroxylated PAH metabolites needs the derivatization step to convert them into non-polar and volatile derivatives. However, a drawback of derivatization was reported. Their derivatives are prone to hydrolysis [18].

HPLC-FLD has many advantages - it offers high selectivity and requires simple sample pretreatment. Moreover, it is also available in most laboratories and has a high sensitivity and good specificity for 1-OHP [12-14, 19]. Many previous methods have been improved, resulting in better precision and accuracy. Normally, the urine sample was adjusted to a pH of 5.0 by adding acid and acetate buffer followed by incubation with hydrolyzing enzyme. The hydrolyzed urine sample was cleaned-up using solid phase extraction, mostly C_{18} cartridge, prior to analyzing with HPLC-FLD [12-14, 19].

Unlike high-dose exposure, such as occupational and smoking exposure, environmental exposure requires a method that can detect low levels of urinary 1-OHP. The present study aimed to improve HPLC-FLD with good sensitivity, reproducibility and high efficiency for detecting urinary 1-OHP.

2. MATERIALS AND METHODS

2.1 Reagents and Chemicals

The chemicals and reagents used in the present study were: 1-hydroxypyrene (1-OHP, molecular weight 218.25, purity 97%) and β-glucuronidase from *Helix pomatia* (85,000 units/10mL, Sigma-aldrich, Steinheim, Germany); 1-hydroxypyprene glucuronide (1-OHP-glu, molecular weight 394.37, Laboratory of Dr. Ehrenstorfer, Augsburg, Germany); hydrochloric acid (HCl, Merck, Darmstadt, Germany); ultra-pure water (a Milli Q apparatus Sytem at Research Institute for Health Sciences, Waters, USA); methanol and acetonitrile (J.T. Baker, Phillipsburg, USA) and C_{18} solid phase extraction (SPE) cartridge (C_{18} - LP tube 300 mg, Vertipak, Nanthaburi, Thailand).

Stock solutions of 1-OHP and 1-OHP-glu were prepared to 1 μg/mL in acetonitrile and kept at -20 °C in the freezer.

Pooled urine samples were prepared by collecting spot-urine samples from five volunteers in the laboratory during working hours on the same day. This pooled urine sample was prepared anonymously and tested for background 1-OHP concentrations. The low background pooled urine was then aliquoted and used for preparing the extraction calibration curve, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy tests and quality control (QC) sample.

2.2 Determination of Optimal Condition of Enzymatic Hydrolysis

Condition of enzyme hydrolysis, including amount of enzyme and duration of hydrolysis, was performed as follows. Urine samples containing 1-OHP-glu were prepared at a concentration five times higher than the 95th percentile concentration (1.2 ng/mL) reported in real samples among the U.S. population aged 6 years and older [20]. In other words, pooled urine samples were spiked to produce a final concentration of 1-OHP-glu of 8 ng/mL. Then, the spiked samples were adjusted to pH 5 with 1 M HCl. Then, 10 mL of the pH-adjusted urine samples were aliquoted to test tubes. Two hundred and fifty microliters of 3 M acetate buffer

(pH 5) were added into each test tube. After that, six sets (n=15 for each set) of urine samples were added to 20, 30, 40, 50, 100, and 200 µL of 8,500 units/mL β-glucuronidase, respectively.

To determine the optimal incubation time, the pooled urine samples were prepared as described above. Samples were then incubated for 2, 3, 4, 5, 6, 8, 12, 16, 20 and 24 h at 37 °C in a hot air oven without shaking.

2.3 Determination of Optimal Condition of Solid Phase Extraction

Aliquots of pooled urine samples were processed under the optimal hydrolysis condition as detailed above. The enzyme-hydrolyzed samples were further processed to optimize the SPE condition. Washing organic solutions, i.e., methanol and acetonitrile, were individually prepared at 30, 40, 50, 60 and 70%. C_{18} cartridge were pre-conditioned with 3 mL of methanol and 3 mL of water, respectively. The hydrolyzed urine samples were loaded onto the cartridge using vacuum manifolds at approximately 5 mmHg pressure. After that, the cartridge were washed with 3 mL of washing organic solutions. The retained 1-OHP was then eluted with 1 mL of 100% methanol for 5 times, consecutively. The extracted 1-OHP eluate was dried under a gentle stream of nitrogen gas and re-dissolved in 0.2 mL of methanol. All sample were filtered with 0.2 µm PTFE syringe filters before HPLC analysis.

2.4 HPLC Analysis Condition

Twenty microliters of filtered sample were analyzed by Agilent 1100 HPLC-FLD with autosampler (Agilent Technologies, Palo Alto, USA). The pretreatment column was Zorbax SB-phenyl C_{16}, (7.5 cm × 4.6 mm, Agilent, USA) while the analytical column was RP-Amide Column (Discovery C_{18}, 250 × 4.6 mm, 5 mm, Supelco, Pennsylvania, USA). The mobile phase was methanol-water (85:15 v/v) with a flow rate of 0.8 mL/min. Column temperature was set at 25 °C. Excitation and emission wavelengths were 242 and 388 nm, respectively.

This study used a six-port switching valve for the on-line, column-switching, HPLC system. The procedure for isolating 1-OHP from the urine involved two periods, as shown in Figure 1.

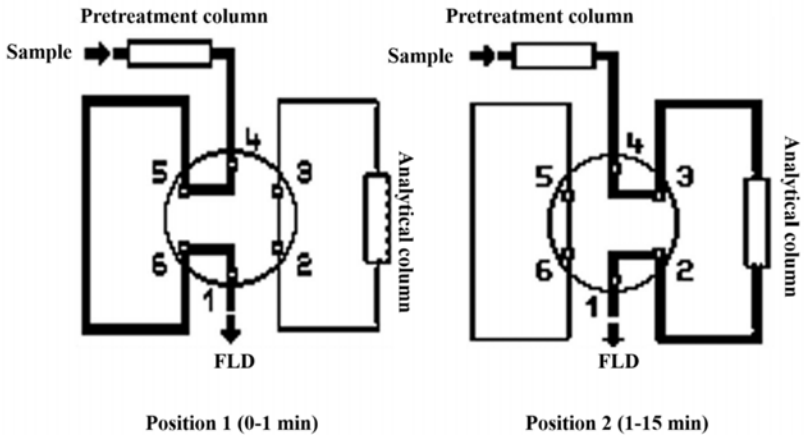

Figure 1. A block diagram of the column-switching HPLC system.

2.5 Method Validation

The linearity of the method was determined by analyzing the pooled urine samples spiked with 1-OHP-glu standard in a total of 10 mL of urine, which yielded 0.06, 0.12, 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, 16.0 and 25 ng/mL (these concentrations derived from dividing 1-OHP-glu concentration by the conversion factor 1.8). The equation of the curve was calculated by a best-fit regression model and the correlation coefficient (r) was used as a measure of the fit of the curve. The equation and correlation coefficient was calculated by Agilent HPLC ChemStation software.

The limit of detection (LOD) and limit of quantification (LOQ) was defined as 3 and 10 times the signal to noise ratio (S/N) of the background levels of these urine samples, respectively [21].

Precision and accuracy were calculated as %relative standard deviation (%RSD) and %recovery, respectively, using spiked pooled urine as quality control (QC) samples. QC samples of 0.06 (very low), 2.0 (low), 8.0 (medium) and 25 (high) ng/mL were prepared by spiking 1-OHP-glu standard solution in pooled urine sample.

LOD, LOQ, precision and accuracy were determined by using matrix match calibration.

This method was externally validated by using proficiency testing materials obtained from German External Quality Assessment Scheme (G-EQUAS, Erlangen, Germany).

2.6 Determination of Urinary Creatinine

Urinary creatinine levels were used to adjust dilution of individual urine samples [22]. The Central Diagnostic Laboratory, Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University, Thailand analyzed creatinine concentration of all urine samples in this study. The urinary 1-OHP concentrations in ng/mL were normalized by creatinine concentrations and expressed as μmol/mol creatinine.

2.7 Application

Urine samples were collected from 130 children aged 10-14 years old in Om Koi District, Chiang Mai Province, Thailand in March 2015. The Human Experimentation Committee, Research Institute for Health Sciences, Chiang Mai University (Project no. 3/58) reviewed and ethically approved this part of the urine study. Spot urine samples, about 30 mL from each individual, were collected at the school. A 1 mL aliquot was taken from each fresh urine sample for determining creatinine. All urine samples were kept at -20 °C until analysis. Urinary 1-OHP levels were analyzed according to the method developed in the present study.

3. RESEULTS AND DISCUSSION

3.1 Optimization of Enzymatic Hydrolysis Condition

Of the various amounts of 8,500 units/mL β-glucuronidase enzyme (20, 30, 40, 50, 100, 200 μL) added to 1-OHP-glu (8 ng/mL), 50 μL (425 units) was adequate to completely deconjugate the 1-OHP-glu at 8 ng/mL in the urine sample (Figure 2).

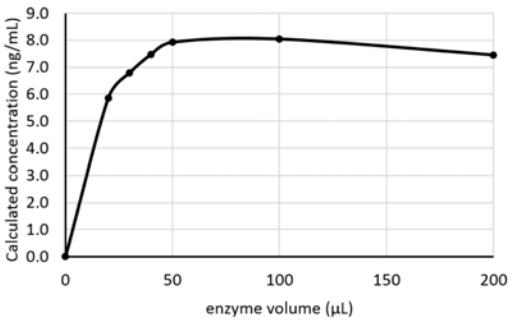

Figure 2. The optimization of enzyme volume for hydrolysis of 8 ng/mL 1-OHP-glu in urine sample.

The hydrolysis efficiency showed an increasing yield with prolonged reaction time. Figure 3 shows the kinetic curve of enzymatic hydrolysis of 8 ng/mL 1-OHP-glu in urine at different incubation times. Percent recovery of 1-OHP, calculated from the ratio of yield and known concentration (8 ng/mL), increased from 25% at the first hour to 90% at the sixteenth hour. After that, the recovery changed slightly. The optimal time of enzymatic hydrolysis was 16 h at 37 °C.

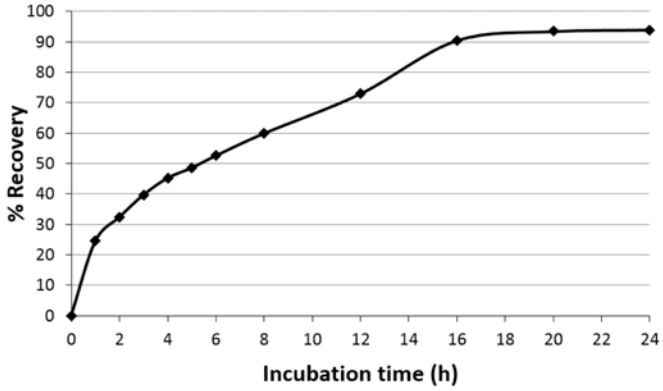

Figure 3. The kinetic curve of enzymatic hydrolysis of 8 ng/mL 1-OHP-glu in urine sample.

3.2 Optimization of SPE Condition

C_{18} solid phase extraction (SPE), used for adsorbing hydrophobic analytes from aqueous solutions, has been used typically for isolating analytes from complex matrices, including urine. Compared to polymeric sorbents, C_{18}-bonded silica sorbent provided good recovery in drug extraction from urine samples [23]. Also, in this study, the absolute extraction recovery of 1-OHP-glu from eight different calibration concentrations were all above 90%.

In comparing washing organic solutions, 30, 40, 50, 60 and 70% of methanol and acetonitrile were used to remove matrix interference from extracted cartridges. The result chromatograms showed that 50% v/v aqueous acetonitrile solution was the most appropriate solution for washing the contaminating compound in the urine matrix from the reversed phase cartridge (Figure 4). Although higher percentages of acetonitrile yielded fewer contaminated peaks, it unnecessarily increases cost and waste. At the same ratio with water, the acetonitrile mixture generally elutes the interference compounds (including phospholipids) from urine better than the methanol mixture. A previous study showed that the recovery of phospholipids and lysophosphatidylcholines decreased with the increasing content of acetonitrile (>50%) on reversed-phase materials [24].

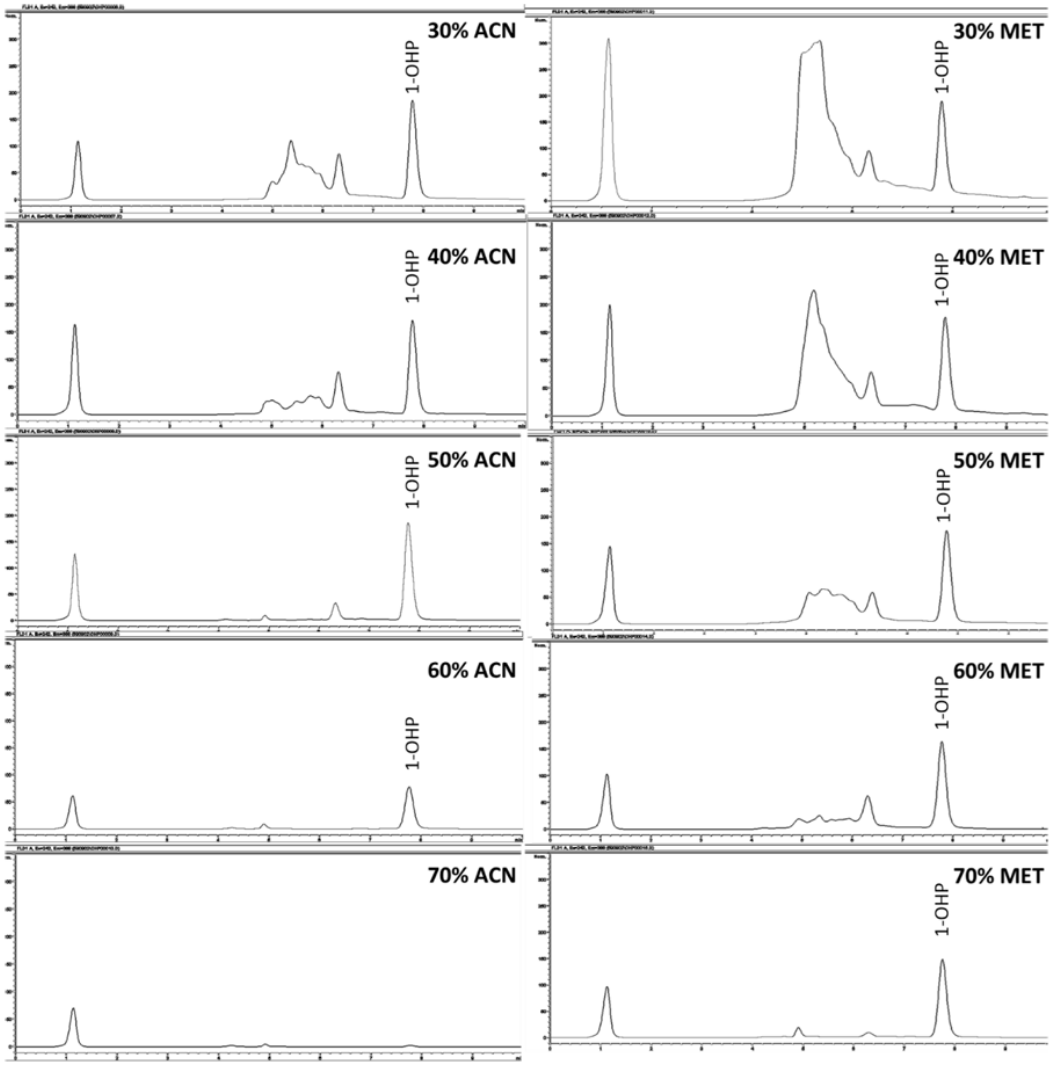

Figure 4. HPLC-FLD chromatograms from different washing solutions at various concentrations. (ACN and MET stand for acetronitrile and methanol, respectively).

3.3 Column-switching Technique

The strategy of column-switching is clearly attractive for analyzing complex mixtures. Although the retention time of the 1-OHP peak shifted, the areas did not differ, as shown in Figure 5. However, the peaks of interference were reduced and yielded a better signal to noise of 1-OHP peak: 11.0 (switching) and 3.20 (non-switching).

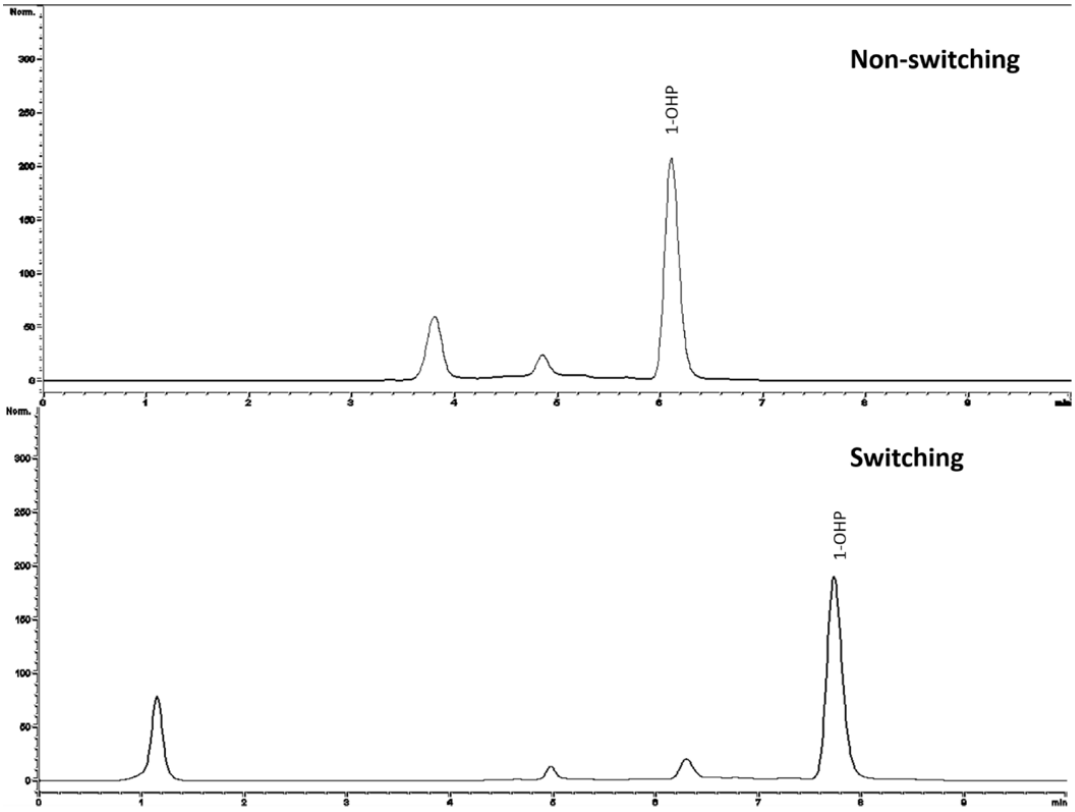

Figure 5. The chromatograms of 1-OHP analysis in pooled urine samples using non-switching and column-switching modes. Signal to noise of 1-OHP peak were 11.0 (switching) and 3.20 (non-switching).

3.4 Method Validation

The method was validated over a dynamic range, owing to the variation in individual measurements of urinary 1-OHP. Because of the wide range of concentration of 1-OHP-glu standard (0.06-25.0 ng/mL), the calibration curve of 1-OHP-glu showed the best fit with nonlinear quadratic regression curves with very good correlation coefficients (r^2= 0.9999). The equation was $y = 0.2665x^2 + 34.27x + 0.3789$. Actually, the linear regression curve (y = 40.38x - 8.480) also fit with good correlation coefficients (r^2= 0.9998), as the same result as previous study [25]. The linear curve in this study over-estimated the concentration at low concentration (0.06 ng/mL) with poor accuracy (% recovery > 120). The broad range of calibration concentrations (0.06-25.0 ng/mL) in this study may produce a nonlinear response, especially at the low and high ends of the range. The typical chromatograms of 1-OHP analysis are shown in Figure 6.

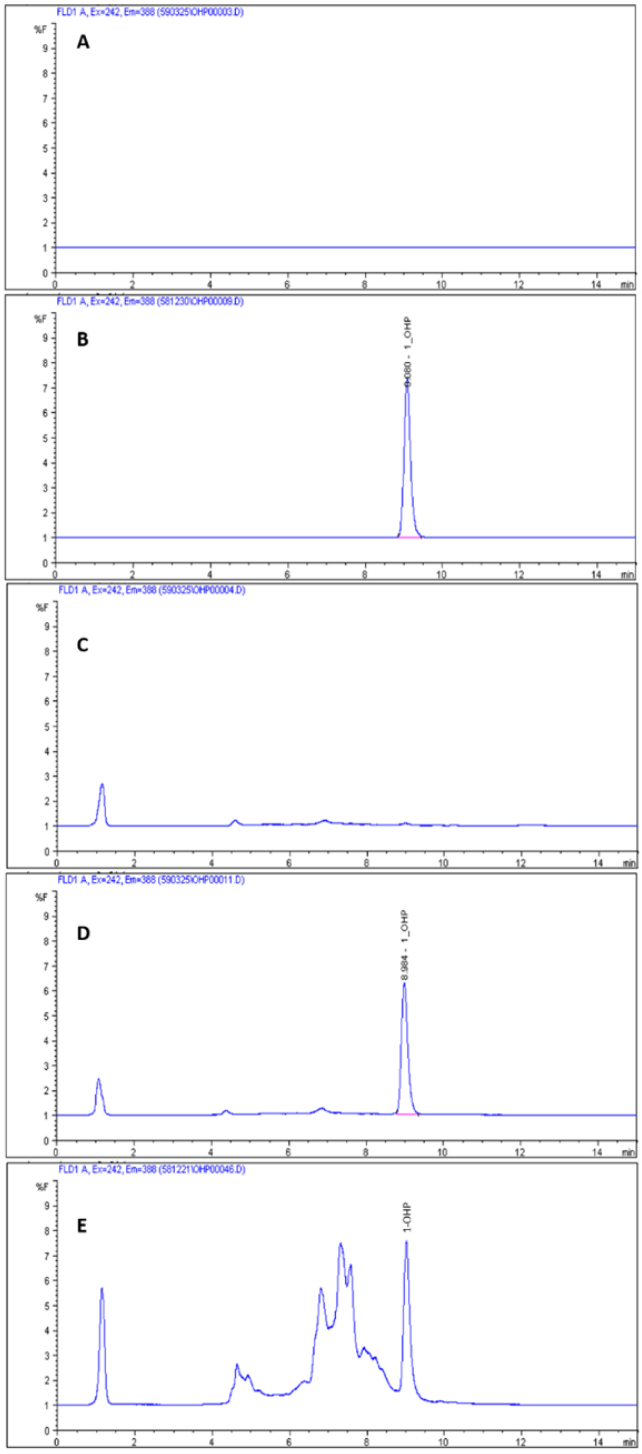

Figure 6. Typical HPLC-FLD chromatograms of (A) methanol, (B) pure free-form 1-OHP standard in methanol, (C) unspiked extracted pooled urine, (D) extracted urine sample spiked with 1-OHP-glu (2.0 ng/mL) and (E) real urine samples (detected concentration at 2.0 ng/mL). All chromatograms are presented in the same Y-axis scale.

The analyte concentration that produced a signal to noise ratio at 3 and 10 was considered as the LOD and LOQ respectively. According to these criteria, the LOD and LOQ of the present method are 0.01 and 0.03 ng/ml

Precision and accuracy were determined from five replicates of pooled urine sample spiked with four different levels - very low, low, medium and high - of 1-OHP-glu (Table 1). At all tested concentrations, intra- and inter-batch precisions were between 1.82-4.22 and 5.01-15.8 %RSD, respectively, which were all within the acceptable limit (< 20 %RSD). Accuracies were between 92.8-117%, which were also all in the acceptable range (80-120%). The calculated %RSD at very low concentration, below 20%, demonstrated that this method had a good reproducibility at the low concentration.

Table 1. Precision, accuracy and extraction recovery of HPLC-FLD method for determining urinary 1-OHP concentration.

Spiked concentrations (ng/mL)	Estimated concentrations (ng/mL)	Precision (%RSD)		Accuracy (%recovery) (n=5)
		Intra-batch (n=5)	Inter-batch (n=5)	
0.06 (very low)	0.07	3.97	15.8	117±5.59
2.0 (low)	2.31	1.84	14.6	111±2.12
8.0 (medium)	7.80	4.22	5.86	92.8±3.92
25 (high)	23.8	1.82	5.01	97.3±1.77

3.5 Comparison with Other HPLC-FLD Methods

Previously reported HPLC-FLD methods, as well as the present method, can detect urinary 1-OHP in very low parts per billion units (ppb, ng/mL) [12-14, 19, 25-27]. Among the methods using enzymatic hydrolysis [13, 14, 19], the present study provides the lowest LOD (0.01 ng/mL), which may have resulted from the optimization of the SPE washing solution. However, compared to other HPLC-FLD methods reported previously, the methods which used magnetic solid phase extraction with specific nanomaterial coating provided the better LOD at 0.001 ng/mL [27]. However, the present method is simple and sensitive enough for detecting in real samples especially from exposure to environmental smoke as shown 100% detection in the result of application.

3.6 Application of the Developed Method on Real Urine Samples

The present developed method was applied for measuring urinary 1-OHP in school children aged 11.5 ±1.87 years (n=130) living in Om Koi District, Chiang Mai Province, Thailand. This district is located in a rural area which the forest fire and open-burning occurs in every dry season. Urinary 1-OHP was detected in all urine samples (100%). The results were shown in both non-adjusted (ng/mL) and creatinine adjusted (µmol/mol creatinine) units.

The concentrations of 1-OHP from all children (100 % detection) ranged from 0.03 to 23.1 ng/mL (Table 2). The means±SD of urinary 1-OHP in these children were 2.08±3.35 ng/mL, or 1.35±2.26 µmol/mol creatinine, which is about seven and twenty times higher than the same aged children living in Bangkok, Thailand (arithmetic mean

0.20 µmol/mol creatinine) [28] and United States (geometric mean 0.01 ng/mL) [10], respectively. Since urine samples were collected during the smoky/hazy period (March), which occurs every dry season in Northern Thailand, high urinary concentrations among the school children in the present study might be caused by exposure to the smoke from biomass burning in this area [29, 30]. This result indicated that the method developed in this present study can detect urinary 1-OHP in real samples from exposure to environmental smoke.

Table 2. Urinary 1-OHP concentrations from 130 children in Om Koi District, Chiang Mai Province, Thailand.

Urinary 1-OHP concentrations		Concentration unit	
		ng/mL	µmol/mol creatinine
Mean		2.08	1.35
Standard deviation		3.35	2.26
Minimum-maximum		0.03-23.1	0.03-16.0
Percentile	5^{th}	0.11	0.07
	25^{th}	0.50	0.26
	50^{th}	0.97	0.55
	75^{th}	2.03	1.46
	95^{th}	7.63	5.58

4. CONCLUSIONS

In conclusion, this developed method presents an improved HPLC-FLD method using column-switching technique for determining urinary 1-OHP and provides low detection limit, good repeatability and high efficiency. In addition, this method is simple and can be applied in wide-range exposure studies including low- and high- dose environmental monitoring in humans.

ACKNOWLEDGEMENTS

We would like to thank all volunteers who participated in this study. This study was supported by Chiang Mai University.

REFERENCES

[1] IARC, Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures; in *IARC Monographs on the Evaluation of Carcinogenic Risk to Humans,* International Agency for Research on Cancer Lyon, France, 2010: **92**.

[2] Li Z., Romanoff L.C., Trinidad D.A., Pittman E.N., Hilton D., Hubbard K., Carmichael H., Parker J., Calafat A.M. and Sjodin A., *Anal. Bioanal. Chem.*, 2014; **406 (13)**: 3119-29. DOI 10.1007/s00216-014-7676-0.

[3] Hu Y., Zhou Z., Xue X., Li X., Fu J., Cohen B., Melikian A.A., Desai M., Tang M., Huang X., Roy N., Sun J., Nan P. and Qu Q., *Biomarkers*, 2006; **11(4)**: 306-18. DOI 10.1080/13547500600626883.

[4] Jongeneelen F.J., *Toxicol. Lett.*, 2014; **231(2)**: 239-48. DOI 10.1016/j.toxlet.2014.05.001.

[5] Zhou Y., Sun H., Xie J., Song Y., Liu Y., Huang X., Zhou T., Rong Y., Wu T., Yuan J. and Chen W., *Am. J. Respir. Crit. Care Med.*, 2016; **193(8)**: 835-46. DOI 10.1164/rccm.201412-2279OC.

[6] Lee M.S., Magari S. and Christiani D.C., *Occup. Environ. Med.*, 2011; **68(7)**: 474-8. DOI 10.1136/oem.2010.055681.

[7] Jain R.B., *Int. J. Environ. Health Res.*, 2016; **26(4)**: 405-419. DOI 10.1080/09603123.2015.1135311.

[8] Khariwala S.S., Carmella S.G., Stepanov I., Fernandes P., Lassig A.A., Yueh B., Hatsukami D. and Hecht S.S., *Head Neck*, 2013; **35**: 1096-1100. DOI 10.1002/hed.23085.

[9] Eom S.Y., Yim D.H., Moon S.I., Youn J.W., Kwon H.J., Oh H.C., Yang J.J., Park S.K., Yoo K.Y., Kim H.S., Lee K.S., Chang S.H., Kim Y.D., Kang J.W. and Kim H., *Anticancer Res.*, 2013; **33**: 3089-3098.

[10] Abid Z., Roy A., Herbstman J.B. and Ettinger A.S., *J. Environ. Public Health*, 2014. Article ID 628508. DOI 10.1155/2014/628508.

[11] Jedrychowski W.A., Perera F.P., Camann D., Spengler J., Butscher M., Mroz E., Majewska R., Flak E., Jacek R. and Sowa A., *Environ. Sci. Pollut. Res. Int.*, 2015; **22(5)**: 3631-3639. DOI 10.1007/s11356-014-3627-8.

[12] Lu S., Fan R., Yu Z., Sheng G. and Fu J., *J. Liquid Chromatogr. Related Technol.*, 2012; **35(11)**: 1528-1537.

[13] Jongeneelen F.J., Anzion R.B.M. and Henderson P.Th., *J. Chromatogr.,* 1987; **413**: 227-232. DOI 10.1016/0378-4347(87)80230-X.

[14] Chetiyanukornkul T., Toriba A., Kizu R., Makino T., Nakazawa H. and Hayakaw K., *J. Chromatogr. A*, 2002; **961(1)**: 107-112. DOI 10.1016/S0021-9673(02)00363-1.

[15] Zhang X., Hou H., Xiong W. and Hu Q., *J. Anal. Methods Chem.*, 2015; **2015**: Article ID 514320. DOI 10.1155/2015/514320.

[16] Holm A., Molander P., Lundanes E., Øvrebø S. and Greibrokk T., *J. Chromatogr. B*, 2003; **794(1)**: 175-183. DOI 10.1016/S1570-0232(03)00427-6.

[17] Campo L., Rossella F. and Fustinoni S., *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, 2008; **875(2)**: 531-540. DOI 10.1016/j.jchromb.2008.10.017.

[18] Gupta M.K., Jain R., Singh P., Ch R. and Mudiam M.K., *J. Anal. Toxicol.*, 2015; **39(5)**: 365-373. DOI 10.1093/jat/bkv023.

[19] Han I.K., Duan X., Zhang L., Yang H., Rhoads G.G., Wei F. and Zhang J., *J. Exp. Sci. Environ. Epidemiol.*, 2008; **18(5)**: 477-485. DOI 10.1038/sj.jes.7500639.

[20] Huang W., Caudill S.P., Grainger J., Needham L.L. and Patterson Jr. D.G., *Toxicol. Lett.*, 2006; **163(1)**: 10-19. DOI 10.1016/j.toxlet.2005.08.003.

[21] UNODC, *Guidance for the Validation of Analytical Methodology and Calibration of Equipment used for Testing of Illicit Drugs in Seized Materials and Biological Specimens,* United Nations Office of Drugs, Vienna, 2009.

[22] Barr D.B., Wilder L.C., Caudill S.P., Gonzalez A.J., Needham L.L. and Pirkle J.L., *Environ. Health Perspect.*, 2005; **113(2)**: 192-200. DOI 10.1289/ehp.7337.

[23] Magiera S., Hejniak J. and Baranowski J., *J. Chromatogr. B*, 2014; **958**: 22-28. DOI 10.1016/j.jchromb.2014.03.014.

[24] Lahaie M., Mess J.N., Furtado M. and Garofolo F., *Bioanalysis*, 2010; **2(6)**: 1011-1021. DOI 10.4155/bio.10.65.

[25] Kuo C.T., Chen H.W. and Chen J.L., *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, 2004; **805(2)**: 187-193. DOI 10.1016/j.jchromb.2003.12.012.

[26] Chen H.W., *Anal. Sci.*, 2007; **23(10)**: 1221-1225. DOI 10.2116/analsci.23.1221.

[27] Huang W., Ding J. and Feng Y.Q., *Chinese J. Anal. Chem.,* 2012; **40(6)**: 830-834. DOI 10.3724/SP.J.1096.2012.11037.

[28] Ruchirawat M., Settachan D., Navasumrit P., Tuntawiroon J. and Autrup H., *Toxicol. Lett.*, 2007; **168(3)**: 200-9. DOI 10.1016/j.toxlet.2006.09.013.

[29] Wiriya W., Prapamontol T. and Chantara S., *Atmos. Res.*, 2013; **124**: 109-122. DOI 10.1016/j.atmosres.2012.12.014.

[30] Kiatwattanacharoen S., Prapamontol T., Singharat S., Somporn Chantara S. and Thavornyutikarn P., *CMU J. Nat. Sci.,* 2017. In press.