Simultaneous biomonitoring of five urinary metabolites of volatile organic compounds in human urine using fast sample preparation method coupled with UHPLC-MS/MS

Karthikeyan Prakasham [1] , Ming-Tsang Wu [1-3] , Po-Chin Huang [1,4] *, Vinoth Kumar Ponnusamy [1,5] *

[1] Ph.D. Program of Environmental and Occupational Medicine, & Research Center for Environmental Medicine, Kaohsiung Medical University (KMU), Kaohsiung City, Taiwan

[2] Department of Public Health, Kaohsiung Medical University, Kaohsiung City-807, Taiwan [3] Department of Family Medicine, KMUH Hospital, Kaohsiung, Taiwan

[4] National Institute of Environmental Health, National Health Research Institute, Miaoli, Taiwan. [5] Department of Medicinal and Applied Chemistry, Kaohsiung Medical University, Taiwan

*Corresponding Authors' E-mail id: kumar@kmu.edu.tw (Prof. Vinoth Kumar) and pchunag@nhri.edu.tw (Dr. PC Huang)

Abstract

Benzene, styrene, ethylbenzene, and vinyl chloride are hazardous volatile organic chemicals produced by gas, paint, rubber, and polyvinyl alcohol industries. The VOCs exposure increases the risk of leukemia, bladder cancer, congenital disabilities, and neurological impairment from the prolonged intake through inhalation and skin contact. The exposed VOCs get activated from CYP2E1 & CYP2B6 enzymes and converted into S-Phenyl mercapturic acid, and t,t muconic from the benzene, phenylglyoxylic acid from ethylbenzene, DL-mandelic acid from styrene, and thiodiglycolic acid from vinyl chloride. Therefore to assess the exposure limits of VOCs have been proposed for the exposed individuals. Therefore in the present study, we developed a novel analytical method for the determination of VOCs' metabolites in human urine using liquid-liquid extraction (LLE) and micro-solid-phase extraction (µSPE) technique coupled with ultra-high-performance liquid chromatography with negative mode tandem mass spectrometry (LC-MS/MS). Target VOCs' metabolites were separated using the C18 stationary phase and 0.4 mL/min mobile phase gradient of 0.1% formic acid in water and methanol. The method limit of quantification ranges between 0.1 ng/mL to 0.5 ng/mL with > 0.992 correlation coefficient for all the metabolites. The method performance shows the extraction recoveries were ranged from 90% to 105% (>7% RSD) with less than 10% of matrix effect. Hence, results conclude that the developed method is highly efficient, sensitive, fast and reliable for the VOCs metabolite analysis. The developed method can be an effective alternative for analyzing VOCs' urinary metabolites for the exposure level identification VOCs.

Keywords: Volatile organic compounds, Biometabolites, Human urine, Biomonitoring, UHPLC-MS/MS.

Highlights: (I) Developed liquid-liquid extraction (LLE) and micro-solid-phase clean-up (µSPE) technique. **(II))** Superior extraction performance with enhanced clean-up efficiency. **(III)** Ecofriendly sample preparation with high sensitive mass detection epidemiology assessment from VOCs.

Introduction

Benzene, styrene, ethylbenzene, and vinyl chloride are IARC classified groups 1 & 2 hazardous volatile organic chemicals produced by gas, petroleum, paint, rubber, and polyvinyl alcohol industries. The emission from the industries leads to air pollution internal and externally and makes humans exposed through inhalation and skin absorption environmentally and occupationally. The exposed VOCs get activated from CYP2E1 & CYP2B6 enzymes and resulting the metabolite formation as shown in figure1. Hence, it increases the risk of leukemia, bladder cancer, congenital disabilities, and neurological impairment. Biological monitoring is essential in assessing human exposure to VOCs by analyzing the respective metabolites and unchanged pollutants in biological samples.

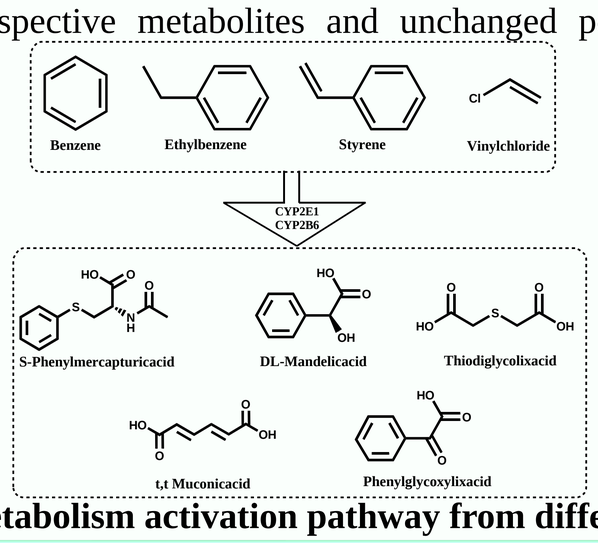

Figure1: Metabolism activation pathway from different enzymes

Objectives

To develop a liquid-liquid extraction (LLE) and micro-solid-phase extraction (µSPE) technique coupled with UHPLC-MS/MS method to identify VOCs' metabolites metabolites in human urine for epidemiological investigations.

Sample preparation

1 mL urine mixed with 1mL1% HoAc in methanol & vortexed for 1 min

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The mixure was allowed to pass by SPE tube (1g$MgSO_4$ + 50 mg C18) and solvent phase was collected

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Collected solvent was concentrated to dryness using N_2 Air @ 35° C

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Dried solution was redissolved in 0.1mL methanol and taken in a HPLC vial

List of chemicals and their properties

S.No	Analyte name	Abbreviation	M.Wt	pKa	M.P	B.P
1	S-Phenylmercapturic Acid	SPMA	239.3	3.18	110-112°C	495
2	t,t-Muconic acid	t,t-MA	142.1	3.77	290	320
3	Thiodiglycolic acid	TDGA	150.1	1.3 , 4.2	128-131	242
4	Phenylglyoxylic acid	PGA	151.1	3.17	62-65	84
5	D,L - Mandelic acid	DL-MA	152.2	3.14	119	322

Table 1: List of chemical studied and their physio chemical parameters

Instrument conditions

UHPLC	Nexera i-series,	MS/MS	8045
Column	C18 (2.7µm)	Ion source (°C)	300 °C
Column oven(°C)	35 °C	DL (°C)	300 °C
Mobile Phase	0.1% HoAc in H_2O & MeOH	Heat block (°C)	350 °C
flow rate	0.4 mL/min	Nebulizing & drying gas	10&3L/min

Table 2: Optimized conditions of UHPLC & MS/MS for VOCs' metabolites

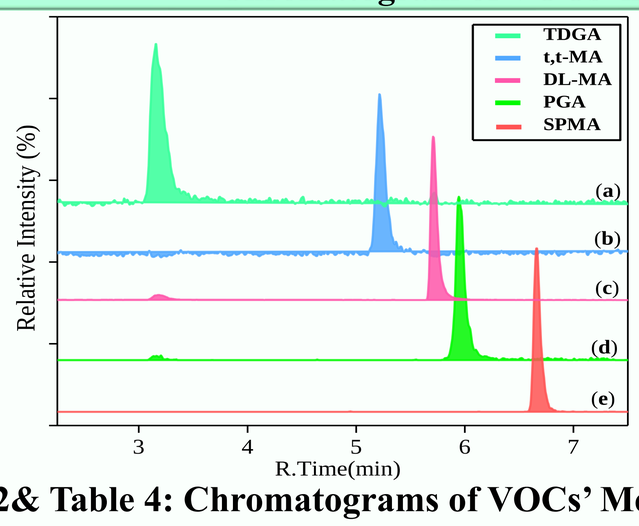
Results and Discussion

Factors influencing the extraction and clean-up process including solvent volume, effect of salt addition, pH were optimized carefully with three replicates. The optimized conditions were evaluated & presented below.

Analyte	Intercept	Slope	R^2	Calib.Range (ng/mL)	LoQ (ng/mL)
Thiodiglycolic acid	-0.0551	0.0024	0.9999	50-10000	50
S-Phenyl mercapturic acid	0.1327	0.0455	0.9998	2-5000	2
Phenylglycolic acid	-0.0053	0.0033	0.999	18.75-3750	18.75
DL- Mandelic acid	0.1252	0.0023	0.9989	25-5000	25
t,t Muconic acid	0.1332	0.0040	0.9995	25-5000	25

Table 3: Method parameters of the newly developed extraction technique

Chromatogram of VOCs' metabolites



Analyte	Precursor & Product ion	CE (V)
TDGA	149.1&105	11
t,t-MA	141&97	10
DL-MA	151&107.1	12
PGA	148.9&77.1	13
SPMA	237.9&109.1	11

Figure 2& Table 4: Chromatograms of VOCs' Metabolites and MRM transitions

Conclusion

A novel method for analyzing urinary metabolites of VOCs in human urine samples based on LLE and µSPE clean-up followed by UHPLC-MS/MS was developed. The sample pretreatment technique allows the extraction in two steps (LLE & µSPE). The method has been checked out in terms of accuracy, precision, recovery, linearity and sensitivity and it has been found that it is applicable in the broad spectrum of VOCs' metabolites in urine samples. The application of the method to urine samples allows to verify its suitability for biomonitoring studies and to glimpse its potential for obtaining valuable exposure data in epidemiological studies.

Acknowledgments

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