Development of Hollow-Fiber Liquid-Phase Microextraction Method for Determination of Urinary *trans,trans*-Muconic Acid as a Biomarker of Benzene Exposure

Libertas Academica
FREEDOM TO RESEARCH

Farhad Ghamari[1], Abdulrahman Bahrami[1], Yadollah Yamini[2], Farshid Ghorbani Shahna[1] and Abbas Moghimbeigi[3]

[1]Excellence Centre of Occupational Health, Research Center for Health Sciences, School of Public Health, Hamedan University of Medical Sciences, Hamedan, Iran. [2]Chemistry Professor, Department of Chemistry, Faculty of Sciences, Tarbiat Modares University, Tehran, Iran. [3]Department of Biostatistics and Epidemiology, School of Public Health and Center of Health Research, Hamedan University of Medical Sciences, Hamedan, Iran.

ABSTRACT: For the first time, hollow-fiber liquid-phase microextraction combined with high-performance liquid chromatography–ultraviolet was used to extract *trans,trans*-muconic acid, in urine samples of workers who had been exposed to benzene. The parameters affecting the metabolite extraction were optimized as follows: the volume of sample solution was 11 mL with pH 2, liquid membrane containing dihexyl ether as the supporter, 15% (w/v) of trioctylphosphine oxide as the carrier, the time of extraction was 120 minutes, and stirring rate was 500 rpm. Organic phase impregnated in the pores of a hollow fiber was extracted into 24 μL solution of 0.05 mol L^{-1} Na_2CO_3 located inside the lumen of the fiber. Under optimized conditions, a high enrichment factor of 153–182 folds, relative recovery of 83%–92%, and detection limit of 0.001 μg mL^{-1} were obtained. The method was successfully applied to the analysis of ttMA in real urine samples.

KEYWORDS: *trans,trans*-muconic acid, benzene exposure, carrier-mediated hollow-fiber liquid-phase microextraction, HPLC-UV, human urine

CITATION: Ghamari et al. Development of Hollow-Fiber Liquid-Phase Microextraction Method for Determination of Urinary *trans,trans*-Muconic Acid as a Biomarker of Benzene Exposure. *Analytical Chemistry Insights* 2016:11 65–71 doi:10.4137/ACI.S40177.

TYPE: Original Research

RECEIVED: May 18, 2016. **RESUBMITTED:** July 10, 2016. **ACCEPTED FOR PUBLICATION:** July 31, 2016.

ACADEMIC EDITOR: Gabor Patonay, Editor in Chief

PEER REVIEW: Three peer reviewers contributed to the peer review report. Reviewers' reports totaled 1398 words, excluding any confidential comments to the academic editor.

FUNDING: This study was part of a Ph.D. thesis at the Hamedan University of Medical Sciences. The authors are grateful to Grant no. 9308203949 for this research. The authors confirm that the funder had no influence over the study design, content of the article, or selection of this journal.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

COPYRIGHT: © the authors, publisher and licensee Libertas Academica Limited. This is an open-access article distributed under the terms of the Creative Commons CC-BY-NC 3.0 License.

CORRESPONDENCE: bahrami@umsha.ac.ir

Paper subject to independent expert single-blind peer review. All editorial decisions made by independent academic editor. Upon submission manuscript was subject to anti-plagiarism scanning. Prior to publication all authors have given signed confirmation of agreement to article publication and compliance with all applicable ethical and legal requirements, including the accuracy of author and contributor information, disclosure of competing interests and funding sources, compliance with ethical requirements relating to human and animal study participants, and compliance with any copyright requirements of third parties. This journal is a member of the Committee on Publication Ethics (COPE).

Published by Libertas Academica. Learn more about this journal.

Introduction

Benzene is an important industrial chemical that has been used in numerous production and synthesis processes in the chemical, petrochemical, paint, plastic, adhesive, rubber, and dye industries. Mineral oil, products of gasoline combustion, and cigarette smoke are the other main sources of widespread environmental and occupational exposure.[1] Exposure to benzene has serious adverse health effects. Long-term exposure to benzene damages the bone marrow and can cause leukemia; benzene exposure is also associated with other blood cancers and precancers of the blood.[2,3] The International Agency for Research on Cancer (IARC) classifies benzene as a group I carcinogen.[4] ttMA, catechol, phenol and hydroquinone, and *S*-phenylmercapturic acid are the biomarkers used for biological monitoring of people exposed to low benzene concentrations (<1).[5,6] The American Conference of Governmental Industrial Hygienists (ACGIH) has recommended ttMA as a biological exposure index of occupational exposure to benzene.[7] It appears that ttMA is a more specific biomarker than other metabolites at high levels of benzene exposure.[8] The ttMA has been found to be a sensitive biomarker for low levels of benzene exposure.[5] Since urinary concentration of ttMA in many subjects exposed to benzene is very low, a reliable means of quantitative analysis, extraction, purification, and preconcentration of the analyte is essential. Several analytical methods, including high-performance liquid chromatography–ultraviolet (HPLC-UV), High Performance Liquid Chromatography—Mass Spectrometry and Liquid Chromatography—Tandem Mass Spectrometry (HPLC/MS/MS), liquid chromatography-tandem mass spectrometry (LC–MS–MS), or gas chromatography mass spectrometry (GC-MS), have been used for the analysis of ttMA in urine.[9–14] When these methods are applied to the determination of ttMA, preconcentration and cleanup steps are required.

Liquid–liquid extraction (LLE) has been used to prepare the samples prior to the analysis of ttMA in biological fluids. In this technique, ethyl acetate or diethyl ether is used as an extraction solvent for sample cleanup. In several studies, solid-phase extraction (SPE) on strong anion-exchange sorbents was used to achieve purification and preconcentration of the ttMA in urine samples.[9–11,15–22]

However, these conventional extraction techniques have disadvantages such as coextraction of interfering substances and

the use of highly toxic and expensive organic solvents. In addition, these techniques are time and labor intensive. In recent years, hollow-fiber liquid-phase microextraction (HF-LPME), a new miniaturized format of LLE, has been developed for separation and preconcentration of target analytes. Its advantages are high selectivity and specificity, low consumption of organic toxic solvents, and low cost. This method is quick, easy, and has no carryover because of the single use of fiber, high preconcentration, and automation.[23–29] In order to promote the extraction of hydrophilic compounds from the donor aqueous phase into the organic phase, carrier-mediated liquid-phase microextraction is used as an active transport mode.[30] In biological and environmental applications, carrier-mediated HF-LPME in its two- and three-phase modes has been used to identify analytes and contaminants such as volatile organic compounds, aromatic amines, herbicides, phenols, pesticides, inorganic compounds, and polar drugs in different matrices.[31–38]

However, till now no studies have been based on application of carrier-mediated HF-LPME technique to the extraction and determination of ttMA in human urine. In this study, carrier-mediated three-phase hollow-fiber microextraction method prior to HPLC-UV was applied to develop a method for extraction, preconcentration, and determination of ttMA in human urine as a biological exposure index for evaluation of environmental and occupational exposure to benzene. Important factors such as the type of organic membrane solvent and carrier concentration, stirring rate, sample and donor pH, sample temperature, and sampling time were optimized, and a simple protocol of HF-LPME method for the assessment of exposure to benzene is represented.

Experimental

Chemicals and reagents. TtMA, tri-*n*-octyl amine (TOA), tributyl phosphate (TBP), trioctylphosphine oxide (TOPO), 1-octanol, o-xylene, methanol, NaOH, HCl, NaCl, Na_2CO_3, and other reagents with analytical grade were purchased from Merck. Dihexyl ether was obtained from Sigma-Aldrich. Deionized water was prepared with a Milli-Q system from Millipore. The Q3/2 Accurel PP polypropylene hollow-fiber (HF) membranes (200 μm wall thickness, 600 μm i.d., 0.2 μm pore size) were obtained from Membrana GmbH.

HPLC system. Separation and determination of the analyte were accomplished on a Knauer HPLC system equipped with a K-2600 variable wavelength UV detector, a K-1001 piston pump (Knauer), an online D-14163 degasser, a 6-port/3-channel injection valve fitted with a 20 μL sample loop and ChromGate software (EZChrom Elite). All injections were performed manually. Chromatographic separations were performed using a C18 analytical column (Eurospher, 25 cm × 4.6 mm i.d., 5 μm).

The isocratic run was performed at a constant flow rate of 1.0 mL minute^{-1}, the mobile phase was water/methanol/acetic acid (69:30:1 (v/v/v)), and the ultraviolet detection wavelength was adjusted at 259 nm with an injection volume of 20 μL.

Supported liquid membrane preparation and extraction procedure. Hollow fibers were manually cut into 8.8 cm pieces with an internal volume of 24 μL, washed with acetone in an ultrasonic bath for five minutes to remove any contaminants and then dried. For each experiment, 24 μL of receiver phase was drawn in to a 25 μL Hamilton HPLC syringe and its needle was inserted into the channel of the hollow fiber. Then, the end of the hollow fiber was soaked in organic solvent for 15 seconds. The hollow fibers were rinsed with distilled water for 10 seconds to remove excess organic solvent from their surfaces. The acceptor phase in the microsyringe was introduced into the lumen of hollow fiber, and one end was sealed with a segment of aluminum foil. During extraction, the membrane portion was immersed in the 11 mL aqueous sample that was contained in a 12 mL sample vial. The sample vial having a 4 mm × 14 mm magnetic stirring bar was placed on a heating magnetic stirrer with 0–1000 rpm (Italy) in a U-shaped configuration. After removing the hollow fiber from the aqueous sample, the end of the hollow fiber was cut and the receiver phase was drawn into the syringe and injected into the HPLC system for analysis. To minimize the memory effect, each piece was used only once.

Preparation of standard solutions and real samples. Stock standard solution of ttMA (1000 μg mL^{-1}) was prepared in HPLC-grade methanol–water (1:1) and stored in a refrigerator at 4°C until use. The working solutions containing analyte of interest were prepared daily by diluting the standard solutions prior to use.

To perform the experimental study, urine specimens were taken from nonsmoking healthy subjects living in a rural area that was not exposed to benzene. Spot urine samples from nine workers who had been occupationally exposed to benzene (in a petrochemical plant, car painting, and gasoline station) were collected. All of the subjects were nonsmoking males with an average age of 43.2 ± 9.7 years and had an average of 13.9 ± 10 years on the job. Urine samples were collected at the end of the shift and at the end of the work week, refrigerated and immediately sent to the laboratory, and kept frozen until analysis. Spot urine samples were collected using 200 mL plastic containers. To protect the containers and specimens from contamination, leak-resistant cups were utilized and each was used only once.

Spiked urine samples with ttMA were made freshly from the stock solution prior to analysis. All subjects gave their written, informed consent to participate in the research, which was conducted in accordance with the principles of the Declaration of Helsinki. The research was approved by the Research Ethics Committee of Hamadan University of Medical Sciences and Health Services.

Results and Discussion

Effect of the carrier and its concentration. An effective organic solvent as a supported liquid membrane should be compatible with hollow fiber, be able to dissolve target

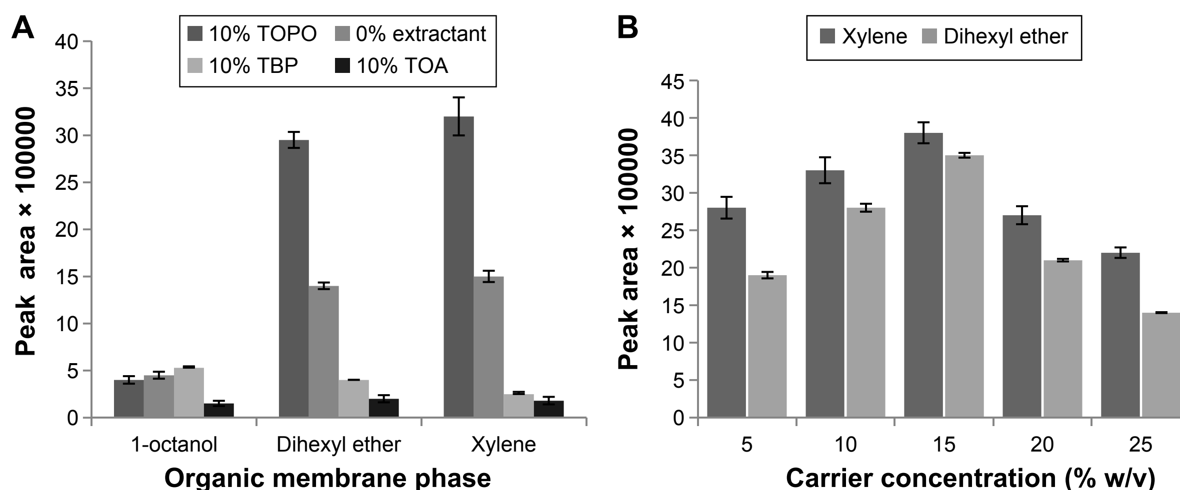

Figure 1. Effects of different carriers (**A**) and carrier concentration (**B**) on extraction efficiency of ttMA by HF-LPME: 11 mL aqueous sample solution of 0.2 µg mL^{-1} metabolite with pH = 3; 24 µL acceptor phase sodium carbonate 0.1 M (pH = 9); stirring rate: 800 rpm; 60 minutes extraction time.

analytes, have a low viscosity to increase mass transfer through the membrane, and low volatility to prevent loss of solvent in the extraction process.

In this paper, three extractants were used as a carrier. Tri-*n*-butyl phosphate (TBP), TOPO, and tri-*n*-octyl amine (TOA) were added to the three organic solvents comprising 1-octanol, dihexyl ether, and *o*-xylene held in the wall pores of the polymer hollow fiber. According to the results, xylene containing 10% TOPO has the highest extraction efficiency (Fig. 1A), but the results of the extractions in different experiments were not the same and its repeatability was not acceptable. Dihexyl ether comprising 10% TOPO was considered for subsequent experiments.

TOPO in xylene showed the best performance efficiency, but not the best extraction efficiency, because the TOPO in xylene was unstable.

In order to improve their extraction efficiency, the solutions of dihexyl ether containing various concentrations of TOPO immobilized in the pores of the porous polypropylene hollow fiber, in the range of 5%–25% (w/v), were investigated.

The best extraction performance was obtained using 15% (w/v) TOPO in dihexyl ether (Fig. 1B). It is assumed that the high dielectric constant or polarity and extremely low solubility in TOPO water are the most important properties and allows for hydrogen bonding between the acidic hydrogen atoms of nonionized ttMA and the phosphoryl oxygen of TOPO to establish efficient extraction of the target analyte into the organic liquid membrane.[39]

Effect of donor pH and acceptor composition. The pH of the aqueous donor and acceptor phase should be adjusted to a value that preserves the analyte in the proper form for improving the extraction recoveries. The ttMA is a compound with a pK_a value of 3.87.[40] It is uncharged in an acidic solution and charged in basic and neutral solutions. In order to investigate the effect of the donor pH on the extraction efficiency, the pH of samples in the 1–3 range was studied. The expected

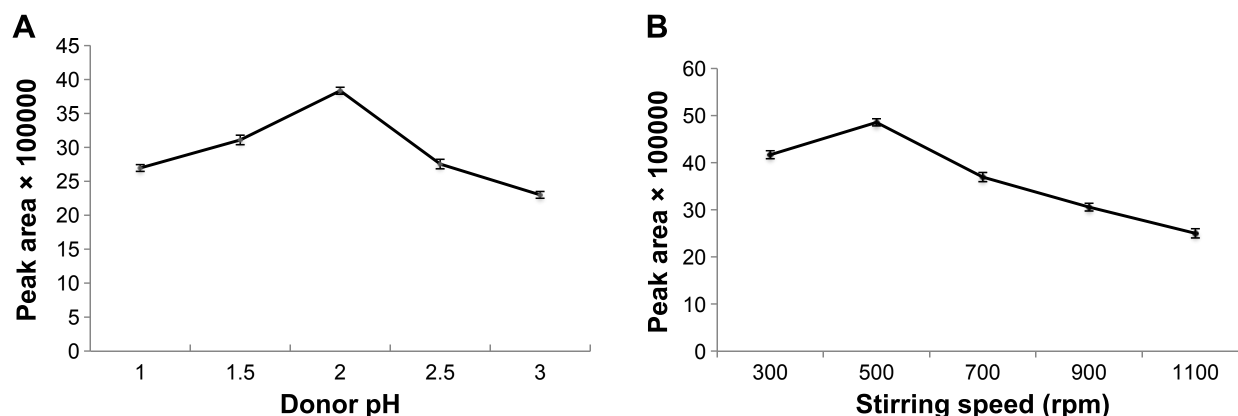

Figure 2. Effects of sample pH (**A**) and stirring speed (**B**) on extraction efficiency of ttMA by carrier-mediated HF-LPME. Extraction conditions: 11 mL aqueous sample solution of 0.2 µg mL^{-1} metabolite; organic membrane phase: 15% TOPO in dihexyl ether; 24 µL acceptor phase sodium carbonate 0.05 M (pH = 8); 60 minutes extraction time.

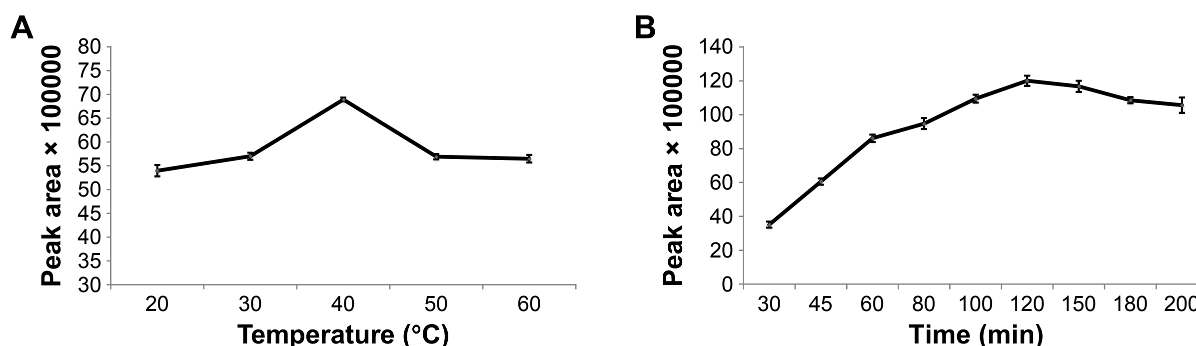

Figure 3. Effects of sample temperature (**A**) and extraction time (**B**) on extraction efficiency of ttMA by carrier-mediated HF-LPME. Extraction conditions: 11 mL aqueous sample solution of 0.2 µL/mL metabolite with pH = 2; organic membrane phase: 15% TOPO in dihexyl ether; 24 µL acceptor phase sodium carbonate 0.05 M (pH = 8); stirring rate: 500 rpm.

pH value was adjusted dropwise by adding concentrated hydrochloric acid into the donor phase. The results revealed that the best extraction was at pH 2.0 (Fig. 2A). Moreover, sodium carbonate in different concentrations of 0.01–2 M was used to determine appropriate stagnant acceptor phase. The best extraction recovery was achieved by using sodium carbonate 0.05 M (pH 8).

Effect of stirring speed. The sample stirring rate in carrier-mediated HF-LPME can increase extraction efficiency so that it promotes mass transfer and diffusion of the analyte from the donor phase into the receiver phase and reduces the time required to reach thermodynamic equilibrium.[41]

In this work, stirring speeds from 300 to 1100 rpm for a 60-minute extraction were studied. The extraction performance increased with stirring rate up to 500 rpm, but decreased at higher rates (Fig. 2B) because high speeds produce air bubbles that cause loss of solvent and reduced precision.

Effect of temperature. Temperature affects the kinetics and the thermodynamics of the distribution process of analytes during extraction and have important effects on the performance of extraction process. Moreover, high temperature increases the loss of organic membrane phase and decreases precision.[31,34,42] In order to observe the effect of temperature on extraction efficiency, the temperatures in the range of 20–60 were studied and 40°C was observed to be the best temperature for the experiments (Fig. 3A).

Effect of extraction time. Liquid-phase microextraction is an equilibrium process. At equilibrium time, analytes in the acceptor phase are in the steady state and stand at their maximum concentration. Moreover, extraction at longer times than the balance time can lead to a decrease in extraction effectiveness because of a loss of organic solvent in pores of hollow fiber or receiving phase.[26,41,43] The peak area in the chromatogram of target analyte increased with extraction time up to 120 minutes. So, a sample extraction time of 120 minutes was chosen for the next study (Fig. 3B).

Method validation and application. Under optimized experimental conditions (15% (w/v), such as TOPO in dihexyl ether as organic liquid membrane, sodium carbonate 0.05 M (pH 8) as acceptor phase, and donor pH 2.0, stirring rate of 500 rpm, 120 minutes as the extraction time, and donor temperature of 40°C, the performance and practical applicability of the three-phase carrier-mediated HF-LPME configuration for extraction of ttMA were investigated. SPE method prior to HPLC-UV analysis was used to confirm the detected analyte according to the validated method of Shahtaheri et al.[9]

Method validation was evaluated on the spiked urine samples comprising the desired amount of the metabolite at various concentrations. Calibration curve of the ttMA was plotted using six spiking samples from 0.005 to 1.2 mg mL^{-1}. To investigate figures of merit of the method, limit of detection (LOD), limit of quantification (LOQ), linearity, linear detection range, repeatability, reproducibility, relative recovery, and enrichment factor (EF) were calculated. The spiked urine calibration curve for ttMA was detected to be linear in the range of 0.005–1.2 µg mL^{-1} with a correlation coefficient (r^2) of 0.996. In this study, to determine LOD and LOQ, spiked urine with known low concentration of ttMA and blank urine

Table 1. Optimization of carrier-mediated HF-LPME coupled with HPLC-UV for quantitative extraction of ttMA in human urine.

CONCENTRATION (µg mL^{-1})	INTRA-DAY RSD% N = 6	INTER-DAY RSD% N = 18	LOD (µg mL^{-1})	DLR (µg mL^{-1})	EF	RR%	R^2
0.01	7.3	8.1					
0.2	6.4	5.6	0.001	0.005–1.2	153–182	83–92	*0.996*
1	2.7	3.4					

Abbreviations: DLR, dynamic linear range; EF, enrichment factor; RR, relative recovery; RSD, relative standard deviation.

samples under optimized experimental conditions were analyzed. The peak-to-peak noise around the metabolite retention time was considered, and then, the concentration of the ttMA that would yield a signal equal to definite value of noise-to-signal ratio was measured. A signal-to-noise ratio (S/N) of 3 was used to calculate LOD and signal-to-noise ratio of 10 was accepted for estimating LOQ. These were found at the ttMA concentration of 0.001 and 0.005 μg mL^{-1}, respectively.

Interday precision was carried out on three consecutive days at three concentrations of the analyte with 18 replications in relative standard deviation (RSD). Intraday precision and accuracy were studied at low, medium, and high concentration levels and with six replications. As shown in Table 1, the RSD% of intra- and interdays were 2.7%–7.3% and 3.4%–8.1%, respectively. The relative recovery, calculated as the ratio of the concentration of the ttMA in the sample solution, was determined with the proposed method to initial concentration of the metabolite in the sample solution, which was 83%–92%. Under optimized conditions, the EF was 153–182 according to the following equation.

$$EF = C_{a,\ final}/C_{a,\ initial} \quad (1)$$

$C_{a,\ final}$ and $C_{a,\ initial}$ are the final metabolites' concentration in the RP and their initial concentration in the donor sample, respectively. The blank urine matrix spiked at three concentrations of the ttMA was used to assess the matrix effects. The matrix effect was established as the ratio of ttMA concentration found in real sample and its concentration in distilled water spiked with the same amount of the analyte. No considerable matrix effects were observed, so it could not have had a significant role on extraction efficiency.

The results of validation experiments indicated that the carrier-mediated three-phase HF-LPME followed by HPLC-UV are suitable and feasible for extraction and analysis of urinary ttMA.

Applicability of the Method for Analysis of Real Samples and Comparison with Other Methods

The proposed carrier-mediated HF-LPME–HPLC-UV method was applied to the biological monitoring of benzene exposure. Nine urine samples were obtained from volunteers at different work environments in Hamedan and Asaluyeh, Iran (collected at the end of the shift and at the end of the work week). The ttMA in these real samples were determined. All of the workers were nonsmoking males with an average age of 43.2 ± 9.7 years and had an average of 13.9 ± 10 years on the job. It was reported that these workers are directly exposed to petroleum products or organic solvent vapors containing benzene in the course of the workday. Before the extraction, the real urine samples were diluted 10-fold with deionized water and, under optimized conditions, extracted and analyzed by using the proposed technique. The results of three replicate analyses of each real urine sample are presented in Table 2. However, a simple dilution of sample prior to analysis is sufficient and desirable when the concentration of the metabolite in the sample is already very high. As illustrated in Table 3, this method is more sensitive and precise than the other published methods (LOD and RSDs). Figure 4 shows typical chromatograms obtained by carrier-mediated HF-LPME for the spiked (0.1 μg mL^{-1}) and real urine samples.

Table 2. Concentrations of ttMA detected in human urine of exposed workers.

NO OF SAMPLE URINE	WORKPLACE	ttMA CONCENTRATION (μg/mL) MEAN ± SD, N = 3
1	Petrochemical plant	1.5 ± 0.13
2	Petrochemical plant	0.7 ± 0.09
3	Petrochemical plant	1.2 ± 0.11
4	Car painting	1.9 ± 0.10
5	Car painting	3.8 ± 0.12
6	Car painting	5.9 ± 0.15
7	Gasoline station	1.5 ± 0.05
8	Gasoline station	0.7 ± 0.07
9	Gasoline station	2.1 ± 0.11

Conclusion

In this work, we presented a new pretreatment method for the quantitative determination of ttMA in urine, which can be used

Table 3. Comparison of the carrier-mediated HF-LPME–HPLC-UV method with other preconcentration techniques for extraction and determination of ttMA in human urine.

EXTRACTION	DETECTION	DETECTION LIMIT (μg mL^{-1})	RSD%	REFERENCES
SPE-derivatization	GC-MS	0.01	7.4	10
SPE	HPLC-UV	0.01	2.7–10	9
SPE	HPLC/MSMS	0.001	3–10	11
LLE	GC-FID	0.02	9.7	16
Filtration	LC–LC–UV	0.05	0.5–5	15
LPME	HPLC-UV	0.001	2.7–7.3	Proposed method

Abbreviations: SPE, solid-phase extraction; LLE, liquid-liquid extraction; LPME, liquid-phase microextraction.

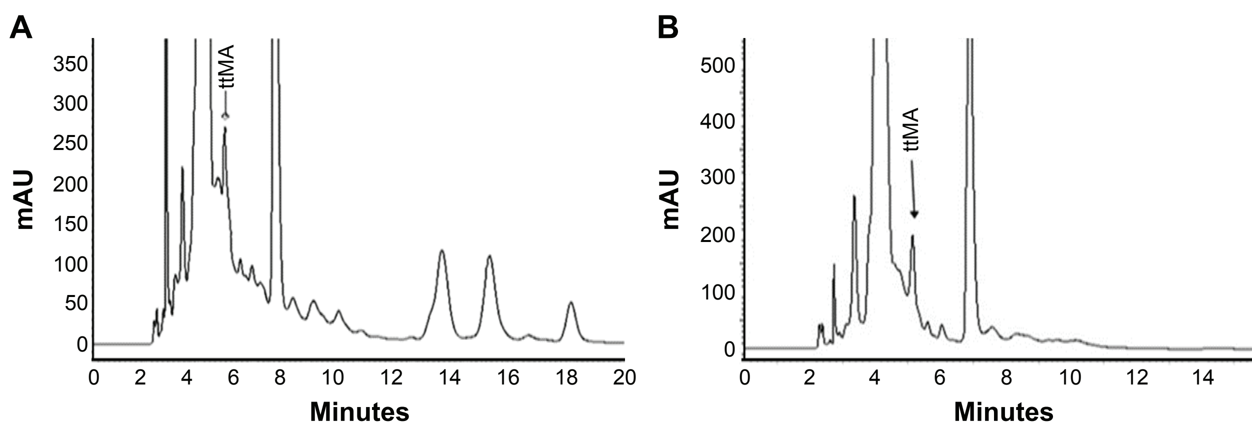

Figure 4. HPLC chromatograms of ttMA after the extraction under optimized conditions as described in the text. (**A**) Spiked urine sample of ttMA at a concentration of 0.1 µg mL^{-1} and (**B**) real urine sample from a petrol station worker exposed to benzene source.

to evaluate occupational and environmental monitoring of exposure to very low concentration of benzene. The factors influencing HF-LPME based on carrier-mediated transport were optimized through this research, concluding that it is an efficient, reliable precision, simple, accurate, and less expensive procedure than conventional extraction methods like LLE, SPE, and derivatization for sample preparation of ttMA as a biomarker of benzene exposure. This method exhibited an EF of around 182, good relative recoveries of 83–92, a very low LOD of 0.001 µg mL^{-1} with wide dynamic linear ranges (0.005–1.2 µg mL^{-1}) and good reproducibility (RSD, less than 8.1%). All these are great advantages over other sample preparation procedures. With this simple extraction procedure, multifold extractions can be performed at the same time with a multistirrer.

Author Contributions

All the authors contributed extensively to the work presented in this article. They discussed the results and implications and commented on the manuscript at all stages. Performed all experiments and wrote the manuscript: FG. Supervised all stages of the project: AB. Designed the experiments and analyzed the data: FGS. Responsible for the chemical aspects of the project: YY. Interpreted the data and performed the statistical analysis: AM. All the authors read and approved the final manuscript.

REFERENCES

1. WHO. International Program on Chemical Safety, Environmental Health Criteria 150: Benzene. Geneva; 1993. Available at: http://www.inchem.org/documents/ehc/ehc/ehc202.htm
2. Rana SV, Verma Y. Biochemical toxicity of benzene. *J Environ Biol*. 2005;26(2):157–168.
3. Arnold SM, Angerer J, Boogaard PJ, et al. The use of biomonitoring data in exposure and human health risk assessment: benzene case study. *Crit Rev Toxicol*. 2013;43(2):119–153.
4. International Agency for Research on Cancer (IARC). *Monographs on the Evaluation of the Carcinogenic Risk of Benzene: Vol. 100F-Evaluation*. Lyon: International Agency for Research on Cancer Research; 2012.
5. Qu Q, Melikian AA, Li G, et al. Validation of biomarkers in humans exposed to benzene: urine metabolites. *Am J Ind Med*. 2000;37(5):522–531.
6. Wiwanitkit V, Suwansaksri J, Nasuan P. Research note: urine *trans*, *trans*-muconic acid as a biomarker for benzene exposure in gas station attendants in Bangkok, Thailand. *Ann Clin Lab Sci*. 2001;31(4):399–401.
7. American Conference of Governmental Industrial Hygienists (ACGIH). TLVs and BEIs: Based on the Documentation of the Threshold Limit Values for Chemical Substances and Physical Agent and Biological Exposure Indices, Cincinnati; 2016.
8. Zhang L, Ye FL, Chen T, Mei Y, Song SZ. *Trans*, *trans*-muconic acid as a biomarker of occupational exposure to high-level benzene in China. *J Occup Environ Med*. 2011;53(10):1194–1198.
9. Shahtaheri SJ, Ghamari F, Golbabaei F. Sample preparation followed by high performance liquid chromatographic (HPLC) analysis for monitoring muconic acid as a biomarker of occupational exposure to benzene. *Int J Occup Saf Ergon*. 2005;11(4):377–388.
10. Ruppert T, Scherer G, Tricker AR, Rauscher D, Adlkofer F. Determination of urinary *trans*, *trans*-muconic acid by gas chromatography-mass spectrometry. *J Chromatogr B Biomed Appl*. 1995;666:71–76.
11. Marchese S, Curini R, Gentili A, Perret D, Rocca LM. Simultaneous determination of the urinary metabolites of benzene, toluene, xylene and styrene using high-performance liquid chromatography/hybrid quadrupole time-of-flight mass spectrometry. *Rapid Commun Mass Spectrom*. 2004;18(3):265–272.
12. Tranfo G, Paci E, Sisto R, Pigini D. Validation of an HPLC/MS/MS method with isotopic dilution for quantitative determination of *trans*,*trans*-muconic acid in urine samples of workers exposed to low benzene concentrations. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2008;867(1):26–31.
13. Chiang W-C, Chen C-Y, Lee T-C, Lee H-L, Lin Y-W. Fast and simple screening for the simultaneous analysis of seven metabolites derived from five volatile organic compounds in human urine using on-line solid-phase extraction coupled with liquid chromatography-tandem mass spectrometry. *Talanta*. 2015;132:469–478.
14. Manini P, De Palma G, Andreoli R, et al. Environmental and biological monitoring of benzene exposure in a cohort of Italian taxi drivers. *Toxicol Lett*. 2006;167(2):142–151.
15. Marrubini G, Hogendoorn EA, Coccini T, Manzo L. Improved coupled column liquid chromatographic method for high-speed direct analysis of urinary *trans*, *trans*-muconic acid, as a biomarker of exposure to benzene. *J Chromatogr B Biomed Sci Appl*. 2001;751(2):331–339.
16. Bartczaka A, Klinead SA, Yua R, et al. Evaluation of assays for the identification and quantitation of muconic acid, a benzene metabolite in human urine. *J Toxicol Environ Health*. 1994;42(3):245–258.
17. Lee BL, Ong HY, Ong YB, Ong CN. A sensitive liquid chromatographic method for the spectrophotometric determination of urinary *trans*, *trans*-muconic acid. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2005;818(2):277–283.
18. Shahtaheri SJ, Abdollahi M, Golbabaei F, Rahimi-Froushani A, Ghamari F. Optimization of SPE for analysis of mandelic acid as a biomarker of exposure to ethyl benzene. *Iran J Environ Health Sci Eng*. 2004;1(2):70–80.
19. Olmos V, Silvia C, Lpez CM, Villaamil EC. High-performance liquid chromatography method for urinary *trans*, *trans* muconic acid. Application to environmental exposure to benzene. *J Anal Toxicol*. 2006;30(4):258–261.
20. Bahrami AR, Joneidi Jafari A, Ahmadi H, Mahjub H. Comparison of benzene exposure in drivers and petrol stations workers by urinary *trans*,*trans*-muconic acid in west of Iran. *Ind Health*. 2007;45(3):396–401.

21. Hopf NB, Kirkeleit J, Bratveit M, Succop P, Talaska G, Moen BE. Evaluation of exposure biomarkers in offshore workers exposed to low benzene and toluene concentrations. *Int Arch Occup Environ Health*. 2012;85(3):261–271.
22. Tunsaringkarn T, Suwansaksri J, Soogarun S, Rungsiyothin A, Zapuang K, Robson M. Genotoxic monitoring and benzene exposure assessment of gasoline station workers in metropolitan Bangkok : sister chromatid exchange (SCE) and urinary *trans*, *trans*-muconic acid (t, t-MA). *Asian Pacific J Cancer Prev*. 2011; 12:223–227.
23. Hultgren S, Larsson N, Nilsson BF, Jönsson JÅ. Ion-pair hollow-fiber liquid-phase microextraction of the quaternary ammonium surfactant dicocodimethylammonium chloride. *Anal Bioanal Chem*. 2009;393(3):929–937.
24. Ebrahimzadeh H, Asgharinezhad AA, Abedi H, Kamarei F. Optimization of carrier-mediated three-phase hollow fiber microextraction combined with HPLC-UV for determination of propylthiouracil in biological samples. *Talanta*. 2011;85(2):1043–1049.
25. Ebrahimzadeh H, Asgharinezhad AA, Adlnasab L, Shekari N. Optimization of ion-pair based hollow fiber liquid phase microextraction combined with HPLC-UV for the determination of methimazole in biological samples and animal feed. *J Sep Sci*. 2012;35(16):2040–2047.
26. Ben-Hander GM, Makahleh A, Saad B, Saleh MI, Cheng KW. Sequential hollow-fiber liquid phase microextraction for the determination of rosiglitazone and metformin hydrochloride (anti-diabetic drugs) in biological fluids. *Talanta*. 2015;131:590–596.
27. Lee J, Lee HK, Rasmussen KE, Pedersen-Bjergaard S. Environmental and bioanalytical applications of hollow fiber membrane liquid-phase microextraction: a review. *Anal Chim Acta*. 2008;624(2):253–268.
28. Yamini Y, Esrafili A, Ghambarian M. Online injection-based hollow fiber liquid-phase microextraction—high-performance liquid chromatography as a fully automatic sample processing for phthalate esters analysis. *Food Anal Methods*. 2016;9(3):729–737.
29. de Bairros AV, de Almeida RM, Pantaleão L, Barcellos T, e Silva SM, Yonamine M. Determination of low levels of benzodiazepines and their metabolites in urine by hollow-fiber liquid-phase microextraction (LPME) and gas chromatography-mass spectrometry (GC-MS). *J Chromatogr B Analyt Technol Biomed Life Sci*. 2015;975:24–33.
30. Bello-López MÁ, Ramos-Payán M, Ocaña-González JA, Fernández-Torres R, Callejón-Mochón M. Analytical applications of hollow fiber liquid phase microextraction (HF-LPME): a review. *Anal Lett*. 2012;45(8):804–830.
31. Ebrahimzadeh H, Yamini Y, Ara KM, Kamarei F. Three-phase hollow fiber microextraction based on carrier-mediated transport combined with HPLC-UV for the analysis of dexamethasone sodium phosphate in biological samples. *Anal Methods*. 2011;3(9):2095–2101.
32. Gao L, Jönsson JÅ. Determination of melamine in fresh milk with hollow fiber liquid phase microextraction based on ion-pair mechanism combined with high performance liquid chromatography. *Anal Lett*. 2012;45(16):2310–2323.
33. Hu XL, Huang YJ, Tao Y, Yin DQ, Liu JF. Hollow fiber membrane supported thin liquid film extraction for determination of trace phenoxy acid herbicides and phenols in environmental water samples. *Microchim Acta*. 2010; 168(1–2):23–29.
34. Farahani H, Yamini Y, Shariati S, Khalili-Zanjani MR, Mansour-Baghahi S. Development of liquid phase microextraction method based on solidification of floated organic drop for extraction and preconcentration of organochlorine pesticides in water samples. *Anal Chim Acta*. 2008;626(2):166–173.
35. Saleh A, Yamini Y, Faraji M, Shariati S, Rezaee M. Hollow fiber liquid phase microextraction followed by high performance liquid chromatography for determination of ultra-trace levels of Se(IV) after derivatization in urine, plasma and natural water samples. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2009; 877(18–19):1758–1764.
36. Tao Y, Liu JF, Hu XL, Li HC, Wang T, Jiang GB. Hollow fiber supported ionic liquid membrane microextraction for determination of sulfonamides in environmental water samples by high-performance liquid chromatography. *J Chromatogr A*. 2009;1216(35):6259–6266.
37. Trtic-Petrovic T, Dordevic J, Dujakovic N, Kumric K, Vasiljevic T, Lausević M. Determination of selected pesticides in environmental water by employing liquid-phase microextraction and liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem*. 2010;397(6):2233–2243.
38. Ma X, Huang M, Li Z, Wu J. Hollow fiber supported liquid-phase microextraction using ionic liquid as extractant for preconcentration of benzene, toluene, ethylbenzene and xylenes from water sample with gas chromatography-hydrogen flame ionization detection. *J Hazard Mater*. 2011;194:24–29.
39. Norberg J, Tiruye D. Supported liquid membrane extraction of urinary *trans, trans*-muconic acid, a biomarker for benzene exposure short communication. *J Sep Sci*. 2002;25:351–355.
40. Yudthavorasit S, Chiaochan C, Leepipatpiboon N. Simultaneous determination of multi-class antibiotic residues in water using carrier-mediated hollow-fiber liquid-phase microextraction coupled with ultra-high performance liquid chromatography tandem mass spectrometry. *Microchim Acta*. 2010;172(1–2):39–49.
41. Esrafili A, Yamini Y, Ghambarian M, Shariati S, Moradi M. Measurement of fluoroquinolone antibiotics from human plasma using hollow fiber liquid-phase microextraction based on carrier mediated transport. *J Liq Chromatogr Relat Technol*. 2012;35(3):343–354.
42. Liu W, Wei Z, Zhang Q, et al. Novel multifunctional acceptor phase additive of water-miscible ionic liquid in hollow-fiber protected liquid phase microextraction. *Talanta*. 2012;88:43–49.
43. Zhang Z, Zhang C, Su X, Ma M, Chen B, Yao S. Carrier-mediated liquid phase microextraction coupled with high performance liquid chromatography for determination of illicit drugs in human urine. *Anal Chim Acta*. 2008;621(2):185–192.