APPLICATION BRIEF

Liquid Chromatography

AUTHORS

Roberto Troiano
Aristide Ganci
PerkinElmer Italia S.p.A., Milan, Italy

Jamie Foss
PerkinElmer, Inc., Shelton, CT, USA

Dr.ssa Ilaria Tomaciello
Laboratori Analisi CEDAM Italia Srl
Bresso, Milan, Italy

Determination of Volatile Organic Compound (VOC) Metabolites in Urine by HPLC with UV Detection

Introduction

Volatile organic compounds (VOCs) are compounds that can evaporate at ambient temperatures[1]. They are emitted from certain solids or liquids and are prevalent in the environment[1,2]. VOCs are present in all homes and workplaces, originating from many different sources, including household and industrial products, office equipment, hobby supplies, tobacco smoke and vehicle exhaust[1]. In the United States, the major non-occupational source of exposure to harmful VOCs is tobacco smoke[3]. Exposure to VOCs can occur through several pathways, including inhalation, ingestion, and dermal contact[2]. Effects of long-term VOC exposure can include increased risk for leukemia, bladder cancer, birth defects, and neurocognitive impairment[2].

The historical approach used to monitor exposure to VOCs focused on the measurement of parent compounds in either blood or urine, which suffered from several drawbacks[1]. The biological half-lives of VOCs are short, on the order of just a few hours and samples were prone to loss of target analytes through volatilization during collection, storage, and analysis[1]. As such, the last several years has seen the focus shift to monitoring urinary metabolites of VOCs due to their quick metabolism in the body via CYP450 mediated pathways[1]. Some metabolites that are formed after via secondary pathways are specific to certain VOCs[1].

PerkinElmer®
For the Better

Monitoring urinary metabolites of VOCs is common in occupations where there is an increased risk of exposure. Often, in high risk environments, personnel are screened at the start and the end of their shift resulting in the need for high throughput analysis of samples. Here we present a rapid and simple liquid chromatographic method for the analysis of six common VOC metabolites in urine for monitoring occupational exposure to VOCs, specifically styrene, toluene, xylene, and ethylbenzene. The urinary VOC metabolites and their associated parent compounds are listed in Table 1. The method also includes creatinine, which is used to normalize the resulting urinary concentrations of the six metabolites. The structures of all seven analytes are shown in Figure 1.

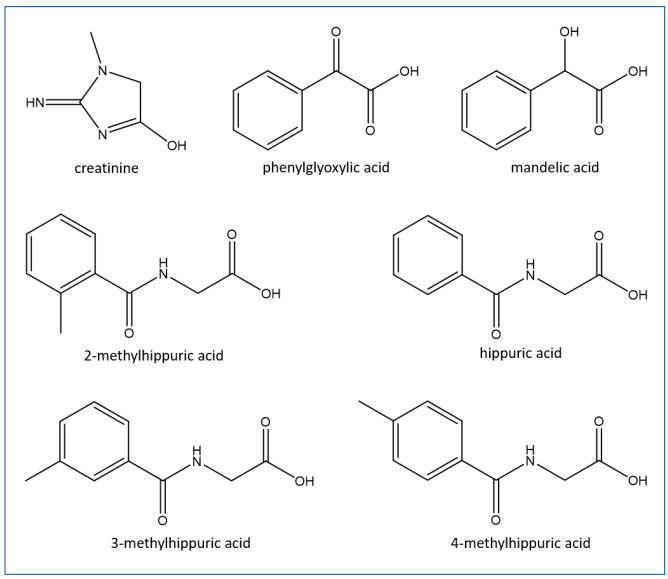

Figure 1: Chemical structures of creatinine and the six VOC metabolites analyzed in this study.

Table 1. Volatile organic compounds and their metabolites.

Volatile Organic Compound (VOC)	Metabolite
Styrene	Mandelic Acid Phenylglyoxylic Acid Hippuric Acid
Toluene	Hippuric Acid
Xylene	2-Methylhippuric Acid 3-Methylhippuric Acid 4-Methylhippuric Acid
Ethylbenzene	Mandelic Acid Phenylglyoxylic Acid

Experimental

Hardware/Software

Chromatographic separation was achieved using a PerkinElmer® LC 300 HPLC system, consisting of an LC 300 HPLC Pump and an LC 300 HPLC Autosampler equipped with an integrated column oven. Detection was achieved using an LC 300 Photodiode Array (PDA) detector. Instrument control, analysis and data processing were performed using the SimplicityChrom™ CDS software platform.

Method Parameters

The LC parameters are shown in Table 2.

Figure 2: LC parameters.

Column	PerkinElmer Epic™ C18, 3 µm, 3.0 x 75 mm (Part# 193191-EC18)
Mobile Phase	See below (Isocratic)
Flow Rate	0.8 mL/min
Analysis Time	12 minutes
Oven Temperature	30 ºC
Sample Temperature	18 °C
Injection Volume	10 µL (Partial loop)
PDA Wavelength	Analytical: 210 nm Bandwidth: 10 nm Reference: 400 nm Bandwidth: 10 nm
Data Collection Rate	5 pts/sec (Hz)
PDA Flow Cell	10 mm (standard)

Solvents, Standards and Samples

All solvents and diluents used were HPLC grade. Unless otherwise specified, standard and sample dilutions were prepared using mobile phase as the diluent.

Mobile Phase Preparation: 1.0 g of 1-octansulphonic acid sodium salt and 2.7 g of monopotassium phosphate (KH_2PO_4) were added to 1L of HPLC grade water and put on a stir plate until fully dissolved. Then 5 mL of 88% conc. o-phosphoric acid and 25 mL of tetrahydrofuran were added. The pH was verified to be between 2.0 and 2.2.

Individual standards were obtained from Sigma-Aldrich®, Inc. to verify analyte retention times. These included creatinine (CREA), mandelic acid (MA), phenylglyoxylic acid (PGA), hippuric acid (HA), 2-methylhippuric acid (2-MHA), 3-methylhippuric acid (3-MHA), and 4-methylhippuric acid (4-MHA). ClinCal® Urine Calibrator (lyophilized) for Occupational Medicine was purchased as the single-point calibration solution from Recipe (Munich, Germany). ClinChek® Urine Control (Level 1) for Occupational Medicine was purchased for use as a quality control sample from Recipe (Munich, Germany).

Standard Solution Preparation: 30 mg of creatinine and 10 mg of each of the VOC metabolite standards were added to a 100-mL volumetric flask. 30-40 mL of acetonitrile was added until full dissolution of the standards was achieved. The solution was then brought up to volume using HPLC grade water, to give a final concentration of 100 mg/L for each of the VOC metabolites and 300 mg/L for creatinine.

Method performance is checked by using the calibrator to quantitate the urine control and verify the results for the control are within the expected range. Both the ClinCal Urine Calibrator and the ClinChek Urine Control Level 1 were prepared following the same procedure used for clinical samples. 20 µL of sample was combined with 1 mL of mobile phase in a 2-mL HPLC vial and vortexed. 10 µL was injected for analysis. For samples that were not clear solutions, they were centrifuged at 4,000 rpm for 5 minutes prior to dilution.

Results and Discussion

The chromatographic separation is demonstrated by the chromatogram of the standard solution shown in Figure 2, with all seven analytes eluting in just over 8 minutes. This method provides good chromatographic resolution between analytes, even providing sufficient resolution of the difficult to separate 3- and 4-methylhippuric acid isomers.

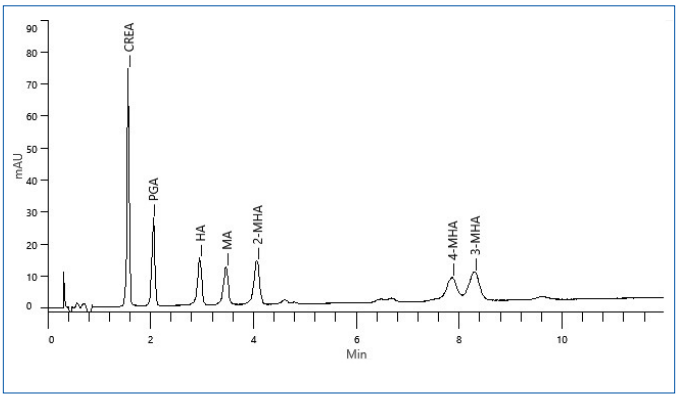

Figure 2: Chromatogram of the standard solution.

It is common in occupational health laboratories to quantitate VOC metabolites in urine samples via single-point calibration using a calibration solution. The chromatogram of the ClinCal Urine Calibrator used in this study can be seen in Figure 3. A control solution is then used to verify the calibration solution to prove the method is suitable for use with real samples. The chromatogram of the ClinChek Urine Control Level 1 solution used in this study can be seen in Figure 4.

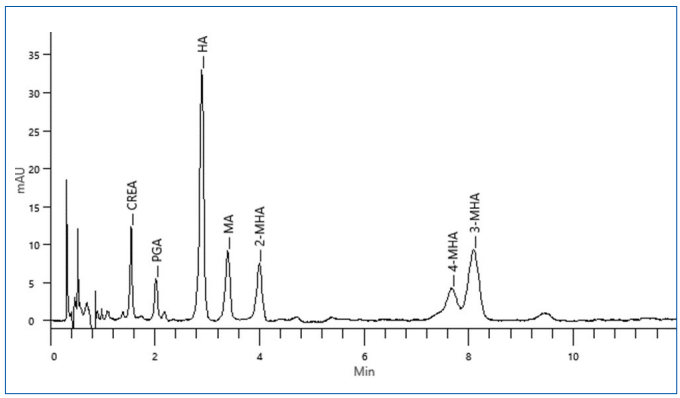

Figure 3: Chromatogram of the ClinCal Urine Calibrator.

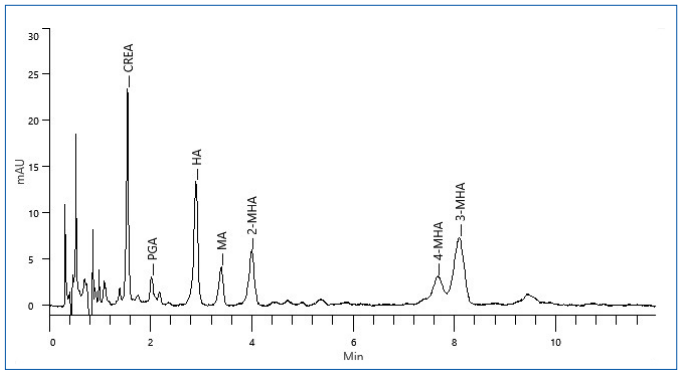

Figure 4: Chromatogram of the ClinChek Urine Control Level 1.

Both the calibrator and control solutions were run in triplicate. The concentrations of each analyte, provided on the Certificate of Analysis (COA) for the ClinCal Urine Calibrator, were used to quantitate the amount of each analyte in the ClinChek Urine Control sample. Results were compared to concentration ranges provided on the COA for the control. As can be seen in Table 2, the results for each analyte in the ClinChek Urine Control sample were within the range specified on the COA, thus demonstrating the suitability of the method to perform the analysis of VOC metabolites in urine.

Figure 3: Results of Control Level 1 sample (analyzed in triplicate).

Analyte	Average, n=3 (mg/L)	Range of Control (mg/L)	In Range?
Phenylglyoxylic acid (PGA)	71.7	49.7 – 74.6	Yes
Hippuric acid (HA)	452.7	340 – 461	Yes
Mandelic acid (MA)	163.4	122 – 183	Yes
2-methylhippuric acid (2-MHA)	191.5	140 – 210	Yes
Sum of 3-methylhippuric acid (3-MHA), 4-methylhippuric acid (4-MHA)	564.4	424 – 635	Yes

Conclusion

This work has demonstrated a simple and rapid chromatographic separation and analysis of six VOC metabolites and creatinine using a PerkinElmer LC 300 HPLC system with PDA detection. A simple, single step dilution of the urine sample in mobile phase prior to HPLC analysis allows for reduced method complexity and increased sample throughput. Following a standard single-point quantitation method using a calibrator solution, the results obtained for a control sample were consistent with values reported on the accompanying COA, demonstrating the suitability of this method to be used for monitoring urinary metabolites of VOCs for workplace exposure.

References

1. Jing Li, A., Kumar Pal, V., Kannan, K. A review of environmental occurance, toxicity, biotransformation and biomonitoring of volatile organic compounds. Environmental Chemistry and Ecotoxicology 2021, 3, 91-116.

2. U.S. Enviromental Protection Agency. A Volatile Organic Compounds Impact on Indoor Air Quality. https://www.epa.gov/indoor-air-quality-iaq/volatile-organic-compounds-impact-indoor-air-quality (accessed September 27, 2021).

3. CDC Environmental Health Laboratory Procedure Manual. (2011-2012). Volatile Organic Compounds (VOCs) Metabolites. Retrieved from https://www.cdc.gov/Nchs/Data/Nhanes/Nhanes_11_12/UVOC_G_MET_VOC_Metabolites.pdfc

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

451377 PKI