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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

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ABSTRACT

Recently, the International Agency for Research on cancer classified outdoor air pollution and particulate matter from outdoor air pollution as carcinogenic to humans (IARC Group 1), based on sufficient evidence of carcinogenicity in humans and experimental animals and strong mechanistic evidence. In particular, a wide variety of volatile organic compounds (VOCs) are volatized or released into the atmosphere and can become ubiquitous, as they originate from many different natural and anthropogenic sources, such as paints, pesticides, vehicle exhausts, cooking fumes, and tobacco smoke. Humans may be exposed to VOCs through inhalation, ingestion, or dermal contact, which may increase the risk of leukemia, birth defects, neurocognitive impairment, and cancer. Therefore, the focus of this study was the development of a simple, effective and rapid sample preparation method for the simultaneous determination of seven metabolites (6 mercaptic acids+*t*,*t*-muconic acid) derived from five VOCs (acrylamide, 1,3-butadiene, acrylonitrile, benzene, and xylene) in human urine by using automated on-line solid-phase extraction (SPE) coupled with liquid chromatography–electrospray tandem mass spectrometry (LC–MS/MS). An aliquot of each diluted urinary sample was directly injected into an autosampler through a trap column to reduce contamination, and then the retained target compounds were eluted by back-flush mode into an analytical column for separation. Negative electrospray ionization tandem mass spectrometry was utilized for quantification. The coefficients of correlation (r^2) for the calibration curves were greater than 0.995. Reproducibility was assessed by the precision and accuracy of intra-day and inter-day precision, which showed results for coefficient of variation (CV) that were low 0.9 to 6.6% and 3.7 to 8.5%, respectively, and results for recovery that ranged from 90.8 to 108.9% and 92.1 to 107.7%, respectively. The limits of detection (LOD) and limits of quantification (LOQ) were determined to within 0.010 to 0.769 ng mL^{-1} and 0.033 to 2.564 ng mL^{-1} in this study. A stability study test included 3 freeze/thaw cycles during short-term storage at room temperature for 36 h and long-term storage at −20 °C for 1 month, and the CV (coefficient of variation) showed less than 8.4, 7.4 and 9.7%, respectively. To the best of our knowledge, this is the first study to provide simple, small injection volumes (40 μL) and a rapid LC–MS/MS method combined with an on-line SPE step for the simultaneous detection, identification, and quantification of seven metabolites derived from five VOCs in human urine for evaluation of the future risk of human exposure to volatile organic compounds.

1. Introduction

Recently, the International Agency for Research on cancer classified outdoor air pollution and particulate matter from outdoor air pollution as carcinogenic to humans (IARC Group 1), based on sufficient evidence of carcinogenicity in humans and experimental animals and strong mechanistic evidence [1]. Air pollutants belong to diverse groups of chemical compounds that are generally grouped into four categories: gaseous pollutants (volatile organic compounds (VOCs), including benzene); persistent organic compounds (polycyclic aromatic hydrocarbons (PAHs), including benzo[a]pyrene (BaP); heavy metals; and, particulate matter (PM).

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In particular, volatile organic compounds (VOCs) are high-production chemicals with several common industrial applications such as chemical intermediates, dry-cleaning chemicals, and solvents, and also are present in cigarette smoke, gasoline, factory fumes and automobile exhaust [2]. According to EPA reports, several VOCs are mutagenic, neurotoxic, genotoxic, and/or carcinogenic and may increase the risk of leukemia, birth defects, neurocognitive impairment, and cancer from both short- and long-term exposure to humans [2–5]. Examples such as benzene and 1,3-butadiene all are proven carcinogens [6]. Acrylamide and acrylonitrile are IARC class 2 carcinogens [7,8]. Xylene has been classified by the IARC as group 3, but it is an important industrial chemical and is a constituent of tobacco smoke [9]. These findings strongly suggest a potential link between exposure to VOCs and diseases in humans.

Over the past few years, exposure to VOCs has often been assessed by the determination of VOC metabolites in urine, and the main advantages of monitoring urinary VOC metabolites are as follows: non-invasive, longer physiological half-life of metabolites compared with parent compounds, and the specificity of mercapturic acid (MAs) metabolites. MAs can be regarded as the final product of the mercapturate metabolic detoxification pathway, and also have been recognized as *N*-acetyl-L-cysteine-*S* conjugates. MAs are released into circulation and removed from the blood by the kidneys for excretion in urine [10,11]. In the present study, we monitored urinary VOC metabolites as biomarkers of exposure to acrylamide, 1,3-butadiene, acrylonitrile, benzene, and xylene. These seven metabolites (Fig. 1) were included: *N*-acetyl-*S*-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA), *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine (DHBMA), *N*-acetyl-*S*-(4-hydroxy-2-buten-1-yl)-L-cysteine (MHBMA3), *N*-acetyl-*S*-(2-cyanoethyl)-L-cysteine (CYMA), trans,trans-muconic acid (*t,t*-MA), *S*-phenylmercapturic acid (PMA), and *N*-acetyl-*S*-(2,4-dimethylphenyl)-L-cysteine (2,4-DPMA).

Various traditional analytical approaches with different sensitivities and specificities can be used to measure VOC metabolites in urine samples. These approaches include gas chromatography with flame ionization detection (GC–FID) [12], gas chromatography with electron capture detection (GC–ECD) [13], gas chromatography with mass spectrometry (GC–MS) [14], high-performance liquid chromatography with ultraviolet absorption detection (HPLC–UV) [15], and high-performance liquid chromatography with fluorescence (HPLC–FD) [16]. All those approaches have specificity for individual VOC metabolites, but a sample preparation protocol such as that used for chemical derivatization reactions is needed or the result could be a lack of sensitivity for low concentrations of VOC metabolites. E-nose analysis of exhaled breath is a new method for assessing VOC profiles in humans [17,18]. Recently, liquid chromatography tandem mass spectrometry (LC–MS/MS) methods have proven to be highly selective, sensitive, accurate, and with the capacity to simultaneously quantify VOC metabolites in human urine [2,11,19,20].

Most published methods for the determination of VOC metabolites in urine involve a labor-intensive and time-consuming procedure that requires purification by solid-phase extraction (SPE) [21–25], such as molecularly imprinted polymers (MIPs) [26] and C18 [27] or liquid–liquid extraction (LLE) [28,29]. To avoid the drawbacks of the extraction method, therefore, we developed a simple, quick and cheap sample preparation method and combined it with on-line SPE-LC–MS/MS, which was rapid, automated, and required only a small sample volume with a decreased matrix effect that provided an accurate, precise and simultaneous determination of these seven metabolites derived from five VOCs (acrylamide, 1,3-butadiene, acrylonitrile, benzene, and xylene.) in human urine samples. Combining these two technologies not only enhanced the signal but also improved the sensitivity compared with other existing methods currently in use. To the best of our knowledge, this is the first study to provide simple, small-injection volumes (40 μL), and a rapid LC–MS/MS method combined with an on-line SPE step that was developed for the simultaneous detection, identification, and quantification of these seven metabolites derived from five VOCs in human urine for an estimation of the possible risk of human exposure to volatile organic compounds.

2. Experimental

2.1. Chemical and reagents

N-acetyl-*S*-(2-hydroxy-3-propionamide)-L-cysteine dicyclohexylammonium salt (GAMA), *N*-acetyl-*S*-(2-hydroxy-3-propionamide)-L-cysteine-d_3 dicyclohexylammonium salt (GAMA-d_3),

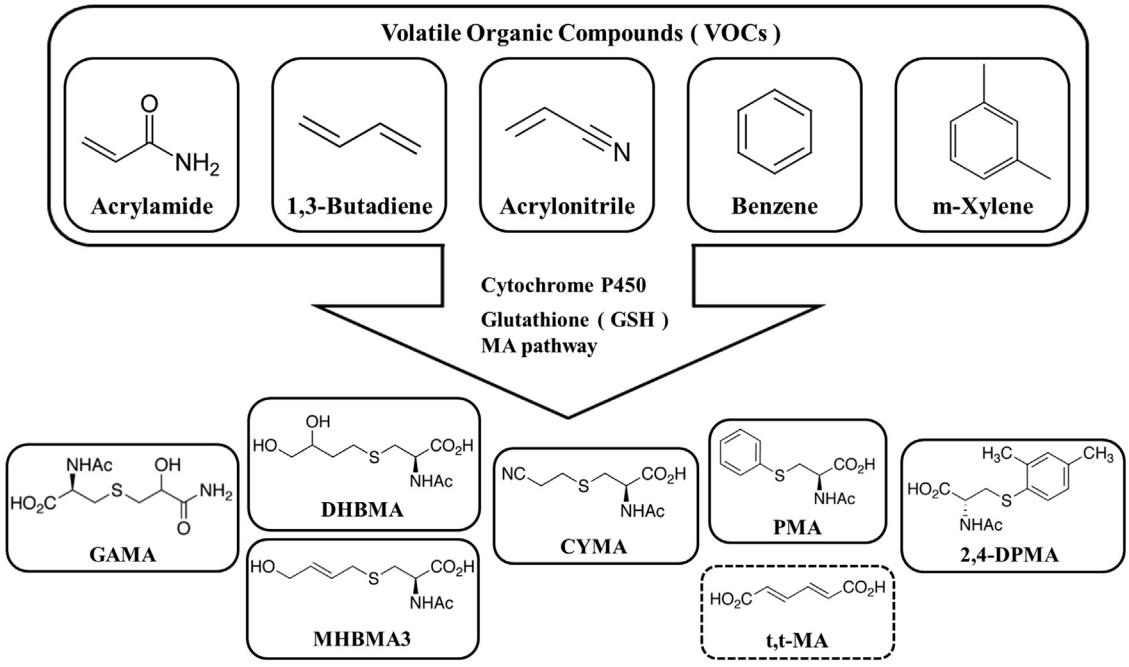

Fig. 1. Metabolic pathway of seven metabolites derived from five VOCs (6 mercapturic acids + *t,t*-muconic acid) in this study.