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High-pressure photon ionization time-of-flight mass spectrometry combined with dynamic purge-injection for rapid analysis of volatile metabolites in urine

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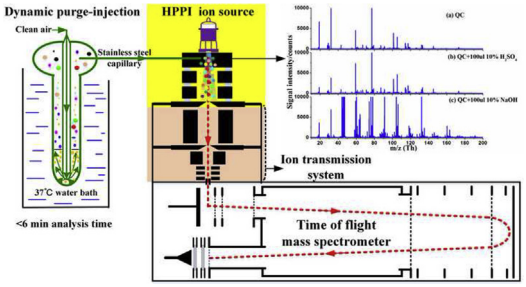
GRAPHICAL ABSTRACT



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ABSTRACT

Small molecule metabolites are widely used as biomarkers in the research field of metabolomics for disease diagnosis and exposure assessment. As a readily available biofluid containing plenty of volatile organic metabolites (VOMs), urine is ideal for non-invasive metabolomic analysis; however, there is still lack of rapid analysis method for VOMs in urine. Here we report a kind of rapid method for urine analysis by employing high-pressure photon ionization time-of-flight mass spectrometry (HPPI-TOFMS) combined with dynamic purge-injection. Various types of metabolites, such as ketones, alcohols, acids, sulfides, pyrroles and amines were detected directly by simple acidification or alkalization of urines. It is noteworthy that nitrogen-containing compounds, especially polar amines, could be ultrasensitively measured without any derivatization. The analytical capability of the direct HPPI-MS technique was demonstrated by analyzing five valuable metabolites, i.e., toluene, 2,5-dimethylpyrrole, trimethlyamine, styrene, and p-xylene, which exhibited relatively low limits of detection, wide linear range and satisfactory repeatability. Being highly sensitive and humidity-friendly, the whole analytical procedure is easily operated in less than 6 min. Interestingly, a new biomarker 2,5-dimethylpyrrole was exclusively found in the smoker's urine sample besides toluene. The work presents a novel tool for rapid nontarget disease biomarkers screening or target monitoring of specific compounds through the investigation of volatile metabolites in urine.

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1. Introduction

Analysis of small molecule metabolites has important significance for the enhancement of biomarker discovery in the research field of metabolomics [1–5]. With the advantages of non-invasive collection, readily available properties, and less complexity with lower protein content, urine is preferred as an ideal biofluid for metabolomic analysis [6–10]. Urinary metabolites could offer specific information for non-invasive screening of human disease such as diabetics and lung cancers [11–14]. In addition, detection of hazadous volatile organic metabolites (VOMs) (toluene, styrene and p-xylene, etc., which exist widely in the atmopshere of industrial manufacture and tobacco smoke) in urine specimen, is important for the assessment of health risks of exposure [15–17]. Although a few gas chromatography-mass spectrometry (GC-MS)-based techniques, such as static or dynamic headspace GC-MS and solid-phase microextraction (SPME) GC-MS, have been utilized for urinary volatiles analysis, however, the long sample pretreatment and analysis time will limit its usage in rapid disease screening field [18–23]. In addition, neither GC nor liquid chromatographic (LC) methods are well-suited for direct and rapid detection of nitrogen-containing compounds such as basic polar aliphatic amines. They are easily retained on the stationary phase of the GC capillary column leading to excessive retention times and poor peak shapes. From another respect, liquid chromatography, hydrophilic interaction chromatography (HILIC-MS) or ion chromatography (IC), may be possible solutions. However, lack of UV-absorption chromophores in the UV spectrum and low sensitivity with most detectors in LC methods, additional prior derivatization step for about 30 min was needed, which increased the complexity and analysis time [24–30].

Direct mass spectrometry, such as selected-ion-flow tube (SIFT) and proton-transfer-reaction (PTR), has been increasingly utilized as one of the powerful techniques for rapid detection of trace volatile organic compounds in a complex matrix. However, few of them have been applied in urine analysis and only headspace sampling combined SIFT-MS has been reported in a few studies [31–34]. The equilibration time should be no less than 5 min in order to increase detection sensitivity. Hence, there is still lack of rapid analysis method for VOMs in urine. Recently, high-pressure photon ionization time-of-flight mass spectrometry (HPPI-TOFMS) has been developed for direct trace gases analysis [35]. The most prominent features of HPPI were the high sensitivity and great tolerance for humidity. Under the condition of 100% relative humidity (37 °C), the achieved limits of detection (LODs) for aliphatic and aromatic hydrocarbons are down to 0.015 ppbv (parts per billion by volume). Moreover, most of the characteristic ions of HPPI were molecular ions or quasi-molecular ions with little clusters, which is beneficial for mass spectral interpretation, and plenty of compounds such as acetone, dimethyl sulfide, isoprene, and phenol, etc., were successfully identified in exhaled breath of healthy individuals. Purge-and-membrane mass spectrometry (PAM-MS) is a technique of combining dynamic purge sampling, membrane extraction and subsequently direct MS detection, which has been utilized for analysis of volatile organic compounds in water and soil samples [36–38]. However, membrane extraction step may pose significant memory effects for some compounds. Therefore, the coupling of dynamic purge-injection sampling and direct MS detection without any other pretreatment process should be an easier and more efficient method for rapid analysis of liquid samples.

In this work, HPPI-TOFMS combined with dynamic purge-injection was developed for sensitive and rapid detection of urinary volatiles. Various categories of VOMs, particularly some nitrogen-containing compounds, were successfully identified, and the methodology performance such as LODs, linear range, intraday and interday repeatability were also explored with illustration of some VOMs. Finally, this new system was applied to the urinary VOMs analysis of healthy nonsmokers as well as smokers as a demonstration of its powerful capabilities in liquid sample analysis.

2. Experimental

2.1. Instrumentation

The detailed description of HPPI-TOFMS has been presented in our previous work and only a brief introduction will be given here [35]. As shown in Fig. 1, HPPI-TOFMS was composed of three major parts: a vacuum ultraviolet (VUV) lamp based HPPI ion source, an ion transmission system, and an orthogonal acceleration TOF (oaTOF) mass analyzer. A VUV krypton lamp (Heraeus Noblelight Ltd., Shenyang, China) with photon energy of 10.6 eV was used as the light source. A high-pressure photon ionization region was constructed by three annular stainless steel electrodes and three polytetrafluoroethylene (PTFE) insulator rings spaced from each other. A stainless steel capillary with 250 μm i.d., and 30 cm length was used as the sampling tube to directly introduce gaseous analytes into the ion source. The ion transmission system, composed of a RF-only quadrupole, a Skimmer-2 electrode, a direct current (DC) quadrupole, and an electrostatic einzel lens, was designed to effectively transfer ions from the ion source into the mass analyzer. The home-built oaTOF mass analyzer had a 401 mm long field-free region, and a mass resolution of 3000 (FWHM) was achieved at $m/z = 78$. The ion signals were recorded using a 100-ps time-to-digital converter (TDC) (model 9353, Ametec Inc., Oak Ridge, U.S.A.) with a repetition rate of 25 kHz.

2.2. Dynamic purge-injection sampling apparatus

The dynamic purge-injection apparatus was composed of a thermostated water bath and a bubbling bottle with 20 ml inner volume, as clearly shown in Fig. 1. The bubbling bottle was consisted of a slim body of glass bottle and a fat capsule with an inner tube. The diameter of the capsule was at least twice bigger than that of the body to prevent any possible foam running into the ion source region. The top of the inner tube was connected with the purge gas, while the bottom of the inner tube with a few small holes (0.1 mm i.d.) was inserted into the bottom of the bottle. After the sample was loaded, the bubbling bottle was sealed with parafilm immediately and incubated in 37 °C water bath for 2 min. Subsequently, a clean air stream (flow rate = 150 ml/min) was purged from the vent of the inner tube into the solutions, where small bubbles were formed. Large quantities of VOMs were released into the gaseous phase by bubbles bursting at the gas-liquid interface, which were taken into HPPI source by stainless steel capillary for analysis. As the sampling flow rate of the capillary was 50 ml/min, the extra gas was exhausted into the atmosphere. The sampling capillary was maintained at 100 °C throughout the whole analysis process to prevent the condensation of the VOMs. Immediately after introduction of the purge gas, data acquisition was started and accumulated for 2 min. The analysis profile was obtained by subtracting background volatiles in the purge-injection bottle from the volatiles measured in urine samples. The whole experiment process, from loading sample to finishing detection, was less than 6 min.