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High-throughput screening and quantitation of guanidino and ureido compounds using liquid chromatography-drift tube ion mobility spectrometry-mass spectrometry

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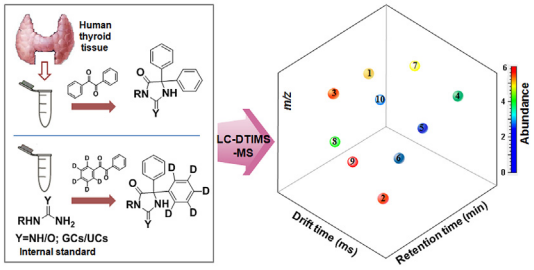
HIGHLIGHTS

- The separation power of DTIMS-MS enhanced peak capacity, spectral clarity, and specificity of benzil labeled GCs and UCs.
- Short-column LC for on-line desalting increased the throughput with a measurement cycle of 5.0 min.
- CCS and accurate mass as a pair of qualifiers were used for the profiling and identification of GCs and UCs.
- An integral abundance arising from 3-D ion features (RT, DT, *m/z*) was used as a novel quantifier for quantitation.
- The developed method was applied to screen and quantify the GCs and UCs in human thyroid tissues.

GRAPHICAL ABSTRACT



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ABSTRACT

The present work focused on the high-throughput screening and quantitation of guanidino compounds (GCs) and ureido compounds (UCs) in human thyroid tissues. The strategy employed benzylic rearrangement stable isotope labeling (BRSIL) for the sample preparation and then detection using liquid chromatography-drift tube ion mobility spectrometry-quadrupole time of flight mass spectrometry (LC-DTIMS-QTOF MS). A short reversed-phase LC realized an on-line desalting and a measurement cycle of 5.0 min. DTIMS separation enhanced the better specificity and selectivity for the benzil labeled GCs and UCs. The elevated mass resolution of QTOF MS enabled measure of the characteristic ions at accurate

Abbreviations: BRSIL, benzylic rearrangement stable isotope labeling; GCs and UCs, guanidino and ureido compounds; LC-DTIMS-QTOF MS, liquid chromatography-drift tube ion mobility spectrometry-quadrupole time of flight mass spectrometry; CCS, collision cross section; IMS, ion mobility spectrometry; IM-MS, ion mobility spectrometry-mass spectrometry; FAIMS, field asymmetric waveform ion mobility spectrometry; RT, retention time; DT, drift time; RP, reversed phase; Cit, citrulline; Arg, L-arginine; Harg, homoarginine; MG, methylguanidine; GBA, 4-guanidinobutyric acid; CT, creatine; M, mol L^{-1}; IS, internal standard; RF, radio frequency; DC, direct current; EIC, extracted ion chromatogram; S/N, signal-to-noise ratio; LOD, limit of detection; LOQ, the limit of quantitation; RSD, relative standard deviation.

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mass in MS and tandem MS spectra. Collision cross section (CCS) from DTIMS and accurate mass from QTOF MS were used as two qualifiers for the profiling and identification of GCs and UCs. In addition, an integral abundance arising from 3-D ion features (retention time, drift time, *m/z*) was applied to quantify the GCs and UCs in human thyroid tissues. The quantitative validation indicated good linearity (coefficient values $\geq$ 0.9981), good precision (1.0%–12.3% for intra-day and 0.9%–7.8% for inter-day) and good accuracy (91%–109%). The results demonstrated that the developed BRSIL coupled with LC-DTIMS-QTOF MS can be a powerful analysis platform to investigate GCs and UCs in human thyroid tissues.

1. Introduction

Ion mobility spectrometry (IMS) enables the gas-phase separation and identification of ionized molecules and has been heavily employed for the detection of explosives, drugs and chemical-warfare agents [1–3]. IMS can be coupled with mass spectrometry (MS), and the combined system (IM-MS) is a technique of growing importance for investigating molecular structures and separating mixtures such as those found in structural chemistry [4], precise intermediates [5–8], proteomics [9–11], glycomics [12,13], and metabolomics [14–16]. It provides valuable analyte structural details that are difficult to obtain using conventional analytical tools through the near-simultaneous acquisition of ion collision cross section (CCS) and ion mass. The resulting two-dimensional correlation spectrum not only presents the structural evidence but also indicates systematical characterization and practical behavior [17,18]. In addition, IM-MS as a hybrid separation technique possesses many of the same advantages of LC-MS approaches, i.e., enhanced dynamic range and increased peak capacity when compared with MS-only analysis [19–21]. Thus, IM-MS has played a significant role in the qualitative analysis.

The capacity of IM-MS for quantitative analysis has also been investigated. Initial applications of IM-MS to quantitative analysis focused on increasing the detection limits of disinfection byproducts in drinking water because this technique improved sensitivity and selectivity by removing interfering signals [22,23]. For example, Ells et al. applied the tandem arrangement of high-field asymmetric waveform ion mobility spectrometry (FAIMS) and MS to the analysis of haloacetic acids [23]. FAIMS demonstrated potential to discriminate haloacetic acids from background ions derived from the electrospray of tap water solutions. IM-MS offers advantages in quantization due to the achievable separation of small molecules, including isomeric molecules, based on their ion mobility differences [24–26]. When molecules cannot be separated by IM directly, the addition of chemical modifiers, such as helium [24], organic modifiers (e.g., methanol, acetonitrile, acetone) [27–29], or alkali metal ions [30] into the drift gas, can result in a substantial improvement in their gas separation. IM-MS can offer a high-throughput quantitative method. The separation in ion mobility space before mass analysis eliminates the need for chromatographic separation [31], allowing for rapid sample processing. This technique is particularly suited for the large number of samples required for the studies on the determination of disease etiology, or the evaluation of drugs and biological agents.

Guanidino compounds (GCs) and ureido compounds (UCs) typically exist at low concentration in biological fluids and play important roles in many biological activities [32,33]. Due to their high polarity, lack of chromophoric groups and water-solubility, various derivatizations combined with spectrophotometry or chromatography were frequently adopted for quantification of GCs [34–36]. MS combined with gas chromatography or liquid chromatography (LC) has been used for the identification and quantification of GCs [37,38]. In contrast, few reports focused on the detection of UCs. Common methods of colorimetric analysis cannot provide the detail of structural information, which may give rise to uncertain results in subsequent analysis. Since GCs and UCs take part in many biochemical processes [39,40], detecting these compounds simultaneously would be of great clinical value. Our previous work has developed a benzylic rearrangement stable isotope labeling (BRSIL) for quantitation of GCs and UCs by LC-ESI MS [41]. Although the identification and quantification of GCs and UCs in biological samples were successful, application of this analysis method in biology and medicine is hampered by the time required.

In this work, a high-throughput analysis of GCs and UCs was explored using LC-drift tube ion mobility spectrometry-quadrupole time of flight mass spectrometry (LC-DTIMS-QTOF MS). LC-DTIMS-QTOF MS can supply retention time (RT), drift time (DT), *m/z* and abundance for each analyte. Reversed phase (RP) LC resulted in short elution time for desalting the samples. DTIMS combined with QTOF MS enabled gas phase separation of analytes for the profiling and identification of GCs and UCs using accurate mass and CCS derived from DT. Furthermore, ion abundance arising from 3-D ion features (RT, DT, *m/z*) was used as a novel quantifier to quantify the GCs and UCs. The quantitative performance of the LC-DTIMS-QTOF MS platform was systematically evaluated and was found to enhance peak capacity, spectral clarity, and specificity compared to analyses performed without IM.

2. Materials and methods

2.1. Chemicals and reagents

Benzil and citrulline (Cit) were purchased from TCI Development Co., Ltd. (Tokyo, Japan). L-Arginine (Arg) and homoarginine (Harg) were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Methylguanidine (MG) hydrochloride, 4-guanidinobutyric acid (GBA) and formic acid were purchased from Sigma-Aldrich (St. Louis, Missouri, U.S.A.). Creatine (CT) was purchased from Alfa Aesar (Haverhill, Massachusetts, U.S.A.). Methanol and dichloromethane were of HPLC grade and were obtained from Merck KGaA (Darmstadt, Germany). All other chemicals and reagents were of analytical grade. Deionized water was produced by a Direct-Q water purification system (Millipore, El Paso, TX, U.S.A.). Benzil-*d5* was synthesized in our laboratory and had been characterized by extensive NMR and MS analysis. Stock solutions of GCs and UCs were prepared in deionized water at a concentration of 0.1 mol L^{-1} (M). Working solutions were obtained by appropriate mixing and dilution of the stock solutions.

2.2. Sample preparation and labeling

Tissue samples were collected from six patients who suffered from thyroid cancer with an informed consent obtained from individual volunteers and ethics approval obtained from Fudan