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Rapid label-free determination of ketamine in whole blood using secondary ion mass spectrometry

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ABSTRACT

A fast and accurate drug screening to identify the possible presence of a wide variety of pharmaceutical and illicit drugs is increasingly requested in forensic and clinical toxicology. The current first-line screening relies on immunoassays. They determine only certain common drugs of which antibodies are commercially available. To address the issue, a rapid screening using secondary ion mass spectrometry (SIMS) has been developed. In the study, SIMS directly analyzed ketamine in whole blood without any pretreatment. While the untreated blood has a complicated composition, principal-components analysis (PCA) is used to detect unknown specimens by building up an analytical model from blank samples which were spiked with ketamine at 100 ng mL^{-1}, to simulate the presence of ketamine. Each characteristics m/z is normalized and scaled by multiplying the root square of intensity and square of corresponding m/z, developed by National Institute of Standards and Technology (NIST). Using linear regression and the result of PCA, this study enables to correctly distinguish ketamine positive and negative groups in an unknown set of specimens. The quantity of ketamine in an unknown set was determined using gas chromatography–mass spectrometry (GC–MS) as the reference methodology. Instead limited by commercially available antibodies, SIMS detects target molecules straight despite the label-free detection capabilities of SIMS, additional data processing (here, PCA) can be used to fully analyse the produced data, which extends the range of analytes of interest on drug screening. Furthermore, extremely low sample volume, 5 μL, is required owing to the high spatial resolution of SIMS. In addition, while the whole blood is analyzed within 3 min, the whole analysis has been shortened significantly and high throughput can be achieved.

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1. Introduction

Fast screening for both legal and illegal drugs has historically been accomplished using immunoassays (e.g. enzyme-linked immuno sorbent assay (ELISA) and enzyme multiplied immunoassay technique (EMIT) [1–3]. They are commonly used as first line screening methods in urine, blood or other bio-fluids [4–7]. The screening is performed with rapid on-site devices based on an immunoenzymatic reaction. Although they are relatively fast and offer high throughput analytical applications, immunoassays are only able to analyze limited common medicinal drugs and drugs of abuse where their antibodies are commercially available [8–10]. Another main deficiency of immunoassays is the cross-reactivity. Compounds with structures similar to the target drug interfere with the results of immunoassays. The amounts of drugs cannot be accurately measured, which leads to prevalence false positive reports or, more importantly, false negative screening results [11–13]. Therefore, a second analytical method, a chromatographic separation (gas chromatography (GC) or liquid chromatography (LC), typically)–mass spectrometry (MS), has been developed either as a complement to immunoassays in clinical testing or as the analytical method in forensic and doping control applications [14–19].

A single MS experiment coupled with GC or LC is able to analyze complex specimens, detect and characterize a large number of known compounds, as well as identify unknown substances. The

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techniques perform high sensitivity and provide well separation. However, majority specimens are subjected to different preparation methods prior to analysis [20–24]. It is usually time consuming and labor intensive. Important missions to be achieved are shortening pretreatment procedure and minimizing sample volume required in both approaches. Alternatively, secondary ion mass spectrometry (SIMS), which sputters a specimen surface by ion bombardment has been emerged to identify target analytes on the biological samples. SIMS is capable of providing lateral resolution and imaging sensitivity on the order of parts-per-million [25–32]. SIMS is regarded as one of the most important microanalytical tools in the semiconductor material industry for analyzing trace amount of dopants and organic impurities. It allows the detection of the elements and small molecular fragments as well as the separation of stable and radioactive isotopes on a surface of materials. Local concentration can also be measured by scanning the ion beam and collecting spectra pixel by pixel with a lateral resolution commonly from 50 nm to 200 nm [29,33,34]. To map a complete surface of complex bio-sample, such as tissues or cells, it takes longer time to obtain enough counts in each pixel. For a homogeneous sample such as blood, an analysis focuses on extracting target analyte ions from a sample rather than profiling its surface, which means only few minutes are required to collect enough data.

With the high-energy ion bombardment, the molecular structure in the surface tends to be broken hence SIMS typically uses isotope or hetero-element labeling to aid the identification of molecules of interest. With the recent development of cluster primary ion such as C_{60}^+, more surface localized interaction and higher sputter rate are obtained and it is possible to generate secondary molecular ions of high mass that identified molecular species directly [35–38]. In other words, label-free analysis of biological and organic surface can be realized. To further enhance the intensity of these molecular ions and hence the sensitivity to molecular information, methods like enhanced oxygen-uptake [39], ion cosputtering [33,40], and optimization of analytical parameters [41] are also being developed.

In the study, C_{60}^+-based molecular SIMS has been developed to determine the administration of ketamine in whole blood. Ketamine, marketed as an anesthetic for human and veterinary, continues to become popular in drug abuse scene. As its self-administration behavior is similar to central nervous system (CNS) depressant drugs, ketamine abuse quickly spreads worldwide. It has been then replaced in the controlled substance in many countries. Recent reports have indicated that ketamine abusers appeared to have severe bladder and kidney damage, such as ulcerative cystitis, severe dysuria, or bladder dysfunction, which are related to the effect of ketamine dose [42–47]. Biological fluids such as urine [48,49] and blood [48,50,51] are commonly used for determining the administration of ketamine. In Taiwan, a cutoff level of 100 ng mL^{-1} is set in human urine. However, there is no criteria for whole blood analysis. Whole blood usually has complex interferences which cause many difficult analytical problems. A sample preparation is required before analysis. Therefore, it is important to develop a rapid and precise screening method. To test the novel technique the same cutoff of ketamine is applied to whole blood analysis here. In the study, extremely low blood volume, 5 μL, was used for detection of ketamine without sample pre-treatment. GC–MS was employed as the reference methodology, for the qualitative confirmation as well as for the quantitative determinations of ketamine. Principal-components analysis (PCA) was performed on characteristic mass fragment intensities from each specimen. With the assistance of PCA, SIMS enabled to characterize the most chemically unique mass fragments of ketamine in complex blood samples and remove much of the ambiguity of similar low mass species that arose from biological samples.

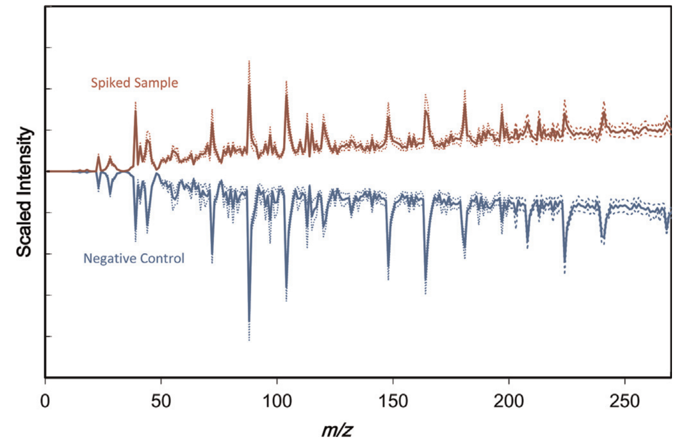

Fig. 1. Scaled spectrum for negative control and spiked sample group. The *y*-axis scaled by multiplying the root square of intensity and square of corresponding *m*/*z* to emphasize the high *m*/*z* region. The broken lines indicated the standard deviation.

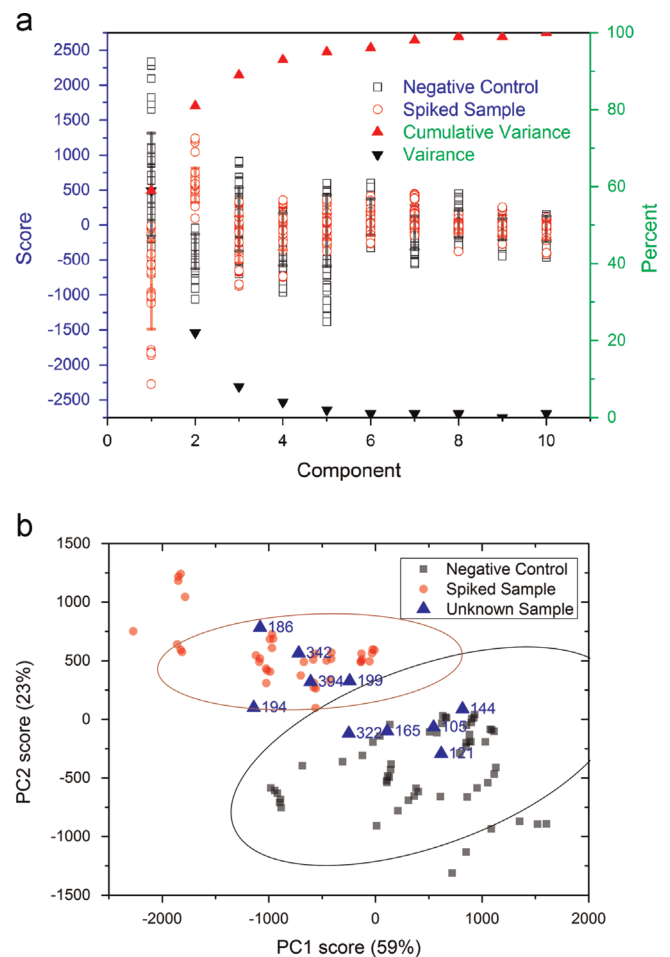

Fig. 2. (a) Distribution of scores for each component (empty symbol) and its percentage in variation (filled symbol). The standard deviation in scores is shown as bars. (b) PCA scores calculated using negative control (square) and spiked sample (circle) projected on PC1 and PC2 space. The elliptical confidence level is calculated using robust method. The scores of unknown samples calculated using linear regression are overlaid as triangles.

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