A Rapid and Sensitive ESI-MS Screening Procedure for Ketamine and Norketamine in Urine Samples*

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Abstract

Traditionally, ketamine was analyzed with gas chromatography (GC) equipped with nitrogen-phosphorus detection, flame-ionization detection, and mass selective detection (MSD) or with liquid chromatography–mass spectrometry (LC–MS). These procedures are sensitive but tedious and slow. There is no commercial immunoassay for ketamine. We have developed a simple and rapid electrospray ionization MS (ESI-MS) procedure to screen ketamine and norketamine (NK) in urine samples. Samples were spiked with ketamine-d₄ (K-d₄) as internal standard and extracted with 0.2 mL of hexane. An experienced technician can prepare a batch of 60 samples in 1 h. An Agilent LC–MSD trap system with autosampler was employed to inject 10-µL extracted samples directly for mass analysis without chromatographic separation. Total analysis time was 1.3 min per sample. The ESI-MS was operated in scan mode. The ion pairs (m/z 238/242 for K/K-d₄ and m/z 224/242 for NK/K-d₄) extracted from the full scan mass spectrum were used for quantification. Because of the nature of the ion trap mass detector employed, the presence of other compounds at high concentration could cause the suppression of target analyte ion intensity determined. Limits of detection were 3 ng/mL for ketamine and 15 ng/mL for NK. Carryover was 0.28% for ketamine and 0.39% for norketamine. Within-run precision (%CV) for K and NK at 3 different concentrations (80, 200, and 600 ng/mL) was 4.0% to 14.7%. A group of 168 urine samples collected from disco-dancing club participants were screened with ESI-MS and confirmed with GC–MS. The sensitivity was 97.1% and specificity was 85.7%. These results indicated that the ESI-MS screening procedure is rapid, sensitive, accurate, and reliable.

Introduction

Ketamine (K) was synthesized by scientists at Parke-Davis Laboratories in 1962 (1) and marketed as an anesthetic drug for human and animal use in the 70s (2). At sub-anesthetic doses, ketamine possesses hallucinogenic properties similar to those of phencyclidine (PCP) (2). It is less toxic than PCP (2). Reports of ketamine abuse appeared soon after its introduction into clinical use (3) and it has since spread worldwide (4–10). A rapid and reliable screening method was needed to identify positive samples before gas chromatographic–mass spectrometric (GC–MS) confirmation. Currently, there is no commercially available immunoassay for ketamine and its metabolites.

Ketamine is metabolized to norketamine (NK) and other metabolites (11). Traditionally, ketamine in biological samples was analyzed with a gas chromatograph equipped with a nitrogen-phosphorus detector (GC–NPD) (6,8,12,13), flame-ionization detector (14), or mass spectrometer (MS) (8–10,15) or with a liquid chromatograph–mass spectrometer (LC–MS) (9). Prior extraction and concentration of ketamine were required before column chromatography–mass spectrometry analysis. These methods are sensitive but time consuming and so are not effective as screening procedures.

Electrospray ionization (ESI)-MS was employed in “screening” procedures for drugs of abuse in many publications (16–18). The reported methods required column chromatography before mass detection. Analysis time was usually long (5 to 30 min). Those methods are not appropriate to serve as screening procedures. The method in the report by Weimann and Svoboda (19) required no column chromatographic separation and serum samples were extracted with solid-phase extraction, then injected directly for mass analysis. The analysis time was only 3 min/sample (19). Zweigenbaum et al. (20) modified the same technique to analyze six benzodiazepines in urine samples. Samples were extracted with organic solvent before analysis. Using 4 autosamplers coupled to 1 chromatographic column and 1 triple-quadrupole MS, 1152 samples were analyzed in less than 12 h. These last two methods employed multiple-reaction monitoring (MRM) mode for mass detection. Both methods are effective as screening procedure for K and NK in urine samples. However, triple-quadrupole MS employed in their study is very expensive, and the technique of Zweigenbaum et al. (20) is complicated.

The ion trap MS has been coupled to GC, LC, and capillary electrophoresis to analyze drugs in urine samples (21–23). Because ion traps measure all ions retained in the trapping steps, they do not suffer the sensitivity losses during the full scan...
mode as compared to triple-quadrupole MS (24). In this report, we employed a less expensive ion trap MS operated in scan mode to screen ketamine and NK in urine samples.

### Materials and Methods

#### Chemicals

Ketamine, NK, and their deuterated analogues ketamine-d$_4$ (K-d$_4$) and norketamine-d$_4$ (NK-d$_4$) were purchased from Cerilliant (Austin TX). Methanol and ethylacetate (EA) were obtained from Mallinkrodt (Paris, KY). Dichloromethane, hexane, toluene, acetonitrile, and isobutanol were obtained from J.T. Baker (Phillipsburg, NJ). Potassium hydroxide, sodium hydroxide, formic acid, and concentrated hydrochloric acid were obtained from Riedel-de Haën (Seelze, Germany). All the organic solvent and chemicals were of the reagent grade.

#### Urine samples

Urine samples were collected from participants suspected of abusing ketamine in a disco-dancing club in Taipei City, Taiwan.

#### Instrument

For the screening experiment, an Agilent (Palo Alto, CA) 1100 series LC-MSD trap was used for mass spectral analysis. An autosampler was employed to inject samples without chromatographic separation. For confirmation, an Agilent GC (6890) coupled to a mass detector (5973) equipped with an autosampler and an HP-5MS capillary column (12.5 m × 0.20-mm i.d., 0.33-µm film thickness, Agilent) was used for GC-MS analysis.

#### Direct liquid injection ESI-MS

**Sample extraction.** To a clean borosilicate glass tube (10 × 75 mm, Kimble) was added 1 mL of urine sample and spiked with internal standard K-d$_4$ (200 ng in 20 µL of methanol). The mixture was alkalinized with 1 mL of 1N sodium hydroxide and extracted with 0.2 mL of hexane by vortex. The mixture was centrifuged at 2000 rpm for 3 min. Eighty microliters of hexane layer was collected for ESI-MS analysis.

**Mass spectral analysis.** The sample injection volume was 10 µL, and the mobile phase constitutes of 5% of A (0.1% of formic acid in deionized water) and 95% of B [toluene in acetonitrile (20:80, v/v)]. Flow rate was 0.4 mL/min. The mass detector parameters were as follows: range, m/z 100 to 250, nitrogen drying gas pressure, 40 psi; flow rate, 5.0 L/min; temperature, 250°C; analysis time, 0.3 min. The extracted ion ratios of m/z 238/242 (K/K-d$_4$) and m/z 224/242 (NK/NK-d$_4$) were used for quantification. Cutoff value is set as 100 ng/mL for K and NK. The injection-to-injection time interval was 1.3 min/sample.

**Carryover rate of ESI-MS.** Seven negative urine samples, one cutoff calibrator, and one sample containing high concentration of K and NK (10 µg/mL) were spiked with K-d$_4$ as internal standard and extracted as in previous section. The sequence of ESI-MS analysis was as follows: seven negative samples (each analyzed once), the cutoff calibrator (analyzed twice), high concentration sample (analyzed twice), and then the same seven negative samples again (analyzed once). Concentration of the seven negative samples was determined before and after the high concentration sample. The increase of the first negative sample following the high concentration sample was used to calculate carryover rate.

#### GC-MS procedure

Detailed procedure was described in a previous publication (15). Briefly, urine samples were spiked with 200 ng of K-d$_4$ and NK-d$_4$ as internal standard. Samples were hydrolyzed with acid and extracted with organic solvent. The organic layer was evaporated to dryness and reconstituted in 100 µL of EA, and 1-3 µL was injected in splitless mode. Selected ion monitoring mode was used for GC–MS analysis. Ions monitored were as follows: m/z 209, 180, 182 for K; m/z 166, 168, 195 for NK; m/z 184, 186, 213 for K-d$_4$; and m/z 170, 172, 199 for NK-d$_4$. Underlined mass fragments were employed for quantification. Cutoff value was set as 100 ng/mL for K and NK.

#### Results

**ESI-MS spectra of K, K-d$_4$, and NK**

Mass spectra of K-d$_4$, K, and NK determined with ESI-MS are shown in Figure 1. Protonated molecular ion (M + H)$^+$ of K-d$_4$, K, and NK (m/z 242, 238, and 224, respectively) were observed. The mass spectra of negative calibrator, cutoff calibrator, and a positive sample are shown in Figure 2. In the presence of high concentration of K and NK was correctly determined.

**Precision and accuracy of ESI-MS procedure**

Calibration curve were generated with different concentrations of K and NK (0, 100, 200, and 500 ng/mL). Linear regression of the calibration curve for K (m/z 238/242) was y = 0.006x + 0.0262, r~$^2$ = 0.9998, for NK (m/z 224/242) was y = 0.001x - 0.0032, r$^2$ = 0.9983. The within-run precision and accuracy for K and NK are presented in Table I. The within-run precision (CV) of three different levels of K and NK at 80, 200, and 600 ng/mL is between 4.0% and 14.7%. The error is between -3.1% and 14.2%. The detection limit (defined as the concentration of sample with signal-to-noise ratio greater than 3) is 3 ng/mL for K and 15 ng/mL for NK.

**Carryover rate of the ESI-MS**

Carryover of the ESI-MS was minimal. For K and NK, the carryover rates were 0.28% and 0.39%, respectively.

**Sensitivity and specificity of the ESI-MS procedure**

In order to determine the sensitivity and specificity of ESI-MS procedure, a group of 168 urine samples collected from disco-dancing club participants were screened with the ESI-MS method. Every sample was also analyzed with GC–MS. Results are shown in Table II. With GC–MS as reference method, there were 68 positive, 84 negative, 2 false-negative, and 14 false-positive samples. Sensitivity (defined as the number of true positive samples divided by the sum of true-positive plus false-negative...
samples) of the ESI-MS procedure is 97.1% and specificity (defined as the number of true-negative samples divided by the sum of true-negative plus false-positive samples) is 85.7%.

Profiles of the discordant samples between ESI-MS and GC–MS

Profiles of discordant samples between ESI-MS and GC–MS are compared and results are shown in Table III. The two false negative samples contained low level of ketamine (81 and 57 ng/mL) and norketamine (344 and 185 ng/mL) by GC–MS. The 14 false-positive samples were divided into two groups, and one group (sample 3 to 11) was screened positive with only low level of K or NK (close to the cutoff concentration). Two of the samples (sample 5 and 10) contained low level of K/NK by GC–MS. The other group of false-positive samples (sample 12 to

Table I. Within-Run Precision and Accuracy of Ketamine and Norketamine Determined with ESI-MS*

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>80</th>
<th>200</th>
<th>600</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precision (CV %)</td>
<td>4.2</td>
<td>5.4</td>
<td>4.0</td>
</tr>
<tr>
<td>Error (%)</td>
<td>0.5</td>
<td>14.2</td>
<td>1.7</td>
</tr>
<tr>
<td>Norketamine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precision (CV %)</td>
<td>7.9</td>
<td>14.7</td>
<td>10.3</td>
</tr>
<tr>
<td>Error (%)</td>
<td>-3.1</td>
<td>9.6</td>
<td>10.7</td>
</tr>
</tbody>
</table>

* Ions monitored: K, m/z 238/242 and NK, m/z 224/242.

Table II. Correlation of the ESI-MS Screening and GC–MS Confirmation Method*

<table>
<thead>
<tr>
<th>GC–MS</th>
<th>ESI–MS†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>68</td>
</tr>
<tr>
<td>–</td>
<td>2</td>
</tr>
</tbody>
</table>

* Cutoff concentration for ESI-MS and GC–MS confirmation is 100 ng/mL of K or NK. Sensitivity: 97.1%, specificity: 85.7%.
† Ions extracted for calculation: K, m/z 238/242 and NK, m/z 224/242.

Table III. Profiles ESI-MS Screening and GC–MS Discordant Samples

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>ESI Screen</th>
<th>GC–MS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K</td>
<td>NK</td>
</tr>
<tr>
<td>Sample #</td>
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<td>2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>0</td>
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</tr>
<tr>
<td></td>
<td>84</td>
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</tr>
<tr>
<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>113</td>
<td>2955</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>760</td>
</tr>
</tbody>
</table>
16) all screened positive with high concentration of NK (760 to 8714 ng/mL) but were tested negative by GC–MS.

Discussion

The rapid increase of ketamine abuse has created a need for a fast and cost-effective procedure to screen out negative samples before confirmation with GC–MS. Immunoassays (IA) with high specificity and sensitivity are the methods of choice to screen drugs of abuse in urine samples (25). However, there was no commercially available IA for ketamine. Traditionally, GC equipped with NPD, FID, and MS were employed to analyze samples with high sensitivity. Those procedures all required prior extraction and concentration of analytes from biological samples before chromatographic separation and quantification. The long analysis time rendered those methods ineffective to serve as screening procedure for multiple samples.

In this report, we presented a simple screening procedure for K and NK. In order to simplify the process, small quantity (0.2 mL) of hexane was used for extraction without further evaporation to dryness. The whole extraction procedure took less than 10 min and used less organic solvent. An experienced technician can process 60 samples in 1 h. The extracted materials were then injected directly into atmospheric pressure electrospray MS. The total analysis time is only 1.3 min, including sample injection. The injection of sample took up most of the time (0.8 min) in the process. The simplified extraction procedure still retain sufficient extraction rate (60% recovery), as compared to regular extraction and concentration procedure (87% recovery). Very high sensitivity was obtained (LOD for ketamine is 3 ng/mL). All samples were also analyzed with GC–MS. Cutoff concentrations for ESI-MS screening and GC–MS confirmation was set at 100 ng/mL of K and NK. Good correlation between ESI-MS and GC–MS was obtained (sensitivity is 97.1%, and specificity is 85.7%).

Although the LOD of our method was less than 20 ng/mL, the cutoff was set at 100 ng/mL, substantially higher than the LOD. This is in accordance with the principle established in the Federal Workplace Drug Testing Program (25). For example, the LODs for amphetamine and methamphetamine were 62.5 ng/mL and 15.6 ng/mL, respectively in one study (26). The cutoff concentration for amphetamine and methamphetamine in GC–MS confirmation assay was set at 500 ng/mL (25).

The two false-negative samples contained only low level of K by GC–MS. The 14 false positive samples were divided into two groups for comparison. One group (sample 3 to 11) was screened positive with concentrations close to the cutoff level. The rest of the false positive samples (sample 12 to 16) were screened false positive with high concentration of K/NK. Those samples were subsequently also found to contain high concentration of MDMA. Because of the nature of the ion trap mass detector employed in this study, suppression of K and K-d4 ions intensity by the more abundant ion of MDMA (m/z 194) was expected. By employing ion ratios of K, NK with K-d4 (internal standard) for quantification, the suppressive effect can be minimized. The mass spectrum of a positive sample presented in Figure 2 indicated that MDMA at concentration greater than 20 µg/mL did not interfere with K/NK determination by ESI-MS. We had experimentally determined that MDMA concentration up to 10 µg/mL did not interfere with ketamine determination. However, at 100 µg/mL of MDMA, serious suppression of target ion intensity was observed. The suppressive effect leads to decrease of target ion intensity to the extent that the observed values are unreliable.

The ESI-MS procedure we described is rapid, sensitive, and specific. It can be employed to screen K and NK in urine samples.

Acknowledgment

This work was supported in part by grant DOH91-NNB-1008 from the Bureau of Narcotic Control, Department of Health, Taiwan and Tzu Chi University Research Fund.

References


Manuscript received November 5, 2003; revision received August 24, 2004.