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A Fast GC-MS Screening Procedure for Ketamine and Its Metabolites in Urine Samples

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ABSTRACT

The rapid increase of ketamine abuse worldwide has created a need for a fast and efficient screening procedure. Currently, there is no commercially available immunoassay. We developed a simple extraction and GC/MS screening procedure to screen out negative samples. Urine samples (1 mL) were spiked with ketamine-d_4 as internal standard. After liquid-liquid extraction, samples were screened for ketamine (K) and its two metabolites, norketamine (NK) and dehydronorketamine (DHNK), with GC/MS without derivatization. The total analytical time is 5 min for each sample. The detection limit for K, NK and DHNK was 10 ng/mL, 10 ng/mL and 30 ng/mL, respectively. The within-run precision and accuracy for 80, 160, 400 ng/mL of K, NK and DHNK were all between -12.50% and 8.17%. We collected 163 urine samples from disco-dancing clubs in Taiwan. Samples were screened and confirmed with GC/MS. The total analytical time for GC-MS confirmation is 20 min per sample. The sensitivity is 98.9% and specificity is 100%. This screening method is rapid, sensitive and applicable to forensic and clinical toxicological analyses.

Key words: fast screen, ketamine, GC-MS

INTRODUCTION

Ketamine, (R, S)-2-(2-chlorophenyl)-2-(methylamino)-cyclohexanone, a derivative of phencyclidine hydrolchloride (PCP), was introduced in the 1960s as a dissociative anesthetic[1]. Like PCP, ketamine interacts with the N-methyl-D-aspartate (NMDA) channel[2]. It is the psychedelic effects that ketamine shares with PCP, including dream-like hallucinations, floating sensations, perceptions of creativity and feeling of arousal[3]. Ketamine is also commonly employed as a veterinary anesthetic.

In addition to its legitimate uses, ketamine is also a drug of abuse commonly identified by street names such as Special K, Vitamin K and K. Along with other drugs such as methylenedioxymethamphetamine (MDMA, Ecstasy) and gamma-hydroxybutyrate (GHB), ketamine has become increasingly popular with the promotion and growth of the rave culture[4,5]. The abuser frequently uses large street doses in an attempt to yield "near-death" experiences[6]. And there were reports of increased abuse of ketamine and ketamine-related deaths[7-9].

Ketamine undergoes an extensive liver metabolism by CYP-450 demethylation to norketamine (NK), followed by dehydrogenation to dehydronorketamine (DHNK)[10]. Some reports suggested that DHNK was an analytical artifact of high-temperature[11,12]. Moore *et al.*[7] used liquid chromatography-mass spectrometry (LC-MS) at low temperature to provide evidence that DHNK is a real metabolite of K. In our previous paper[13], we also used non-derivatized method to analyze K, NK and DHNK to also confirm this point. According to the data of Moore *et al.*, DHNK would be the most sensitive target analyte for potential immunoassay. But to date, there is no commercial immunoassay available.

Here, we developed a simple method to simultaneously screen K, NK and DHNK in urine samples.

MATERIALS AND METHODS

I. *Chemicals*

Ketamine, NK, ketamine-d_4 (K-d_4), and norketamine-d_4 (NK-d_4) were purchased from Cerilliant (Austin TX, USA). DHNK was purchased from Formosa Laboratories (Taiwan). Methanol and ethyl acetate (EA) were purchased from Mallinckrodt, USA. Dichloromethane and isobutanol were purchased from J. T. Baker, USA. Sodium hydroxide, potassium hydroxide, and concentrated hydrochloric acid were purchased from Riedel-deHaën, Germany. All the organic solvents and chemicals are reagent grade.

II. *Urine Samples*

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

III. *Instrument*

A Hewlett Packard GC (6890) coupled to a mass detector (5973) equipped with an autosampler and a HP-

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5MS capillary column (12.5 m × 0.20 mm i.d., 0.33 μm film thickness) (Agilent Technologies, Palo Alto, CA) was used for GC-MS analysis.

IV. *GC-MS Screening Procedure*

To a clean borosilicate glass tube (Kimble 10 × 75 mm), 1 mL of urine sample was added and spiked with internal standard K- d_4 (100 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, then stood for 2 min. Eighty microliter of hexane layer was collected for GC-MS analysis.

Two microliter was injected (splitless). Injection port and interface temperature were maintained at 260˚C and 280˚C, respectively. GC oven was held at 160˚C for 0.08 min then increased to 220˚C at 35˚C/min and held for 0.01 min, then ramped to 260˚C at 40˚C/min, then held at the temperature for 0.01 min. Flow rate was 1 mL/min. Selected ion monitoring mode was used for the analysis. The extracted ion ratios of *m/z* 209/184 (K/K-d_4), *m/z* 166/184 (NK/K- d_4) and *m/z* 153/184 (DHNK/K- d_4) were used for quantification. Cutoff value was set as 100 ng/mL for K, NK or DHNK. Total analysis time was 5 min per sample. There was no solvent blank in between samples.

V. *GC-MS Confirmation Procedure*

Detailed procedure was described in our previous publication[13]. Briefly, urine samples were spiked with 200 ng of K-d_4 and NK- d_4 as internal standards. Samples were extracted with 4 mL of dichloromethane containing 10% isobutanol. 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: K, *m/z* 209, 180, 182; NK, *m/z* 166, 168, 195; K-d_4, *m/z* 184, 186, 213; NK-d_4, *m/z* 170, 172, 199 and DHNK, *m/z* 153, 138, 118. Underlined mass fragments were employed for quantification. Cutoff value was set as 100 ng/mL for K, NK, or DHNK. Total analysis time was 20 min per sample. A solvent blank was run in between samples.

RESULTS

I. *Performance Characteristics of the GC/MS Screening Procedure*

(I) *GC-MS chromatogram*

Total ion chromatogram and the extract ion chromatogram of K-d_4, K, NK, and DHNK with GC-MS screening method were shown in Figures 1 and 2, respectively. The total analytical time is 5.0 min, including the time required for sample injection and for oven to return to initial temperature.

(II) *Precision and accuracy*

The within-run precision (% CV) of the three different control levels (80, 160, 400 ng/mL) of K, NK and DHNK were all less than 8.17%. The error of accuracy was all between –12.5% and 5.75% for the controls. These data were shown in Table 1. Accuracy of the procedure was presented as percent error, calculated as follows: [(measured value - expected value)/ expected value] × 100%.

(III) *Linearity and detection limit*

The linearity of the method was determined with calibration standards at 0, 100, 200, and 500 ng/mL. Linear regression of the calibration curve for K was y = 0.0036x + 0.0046, R^2 = 0.9990, for NK was y = 0.009x + 0.00961, R^2 = 0.9982, and for DHNK was y = 0.0054x + 0.0233, R^2 =

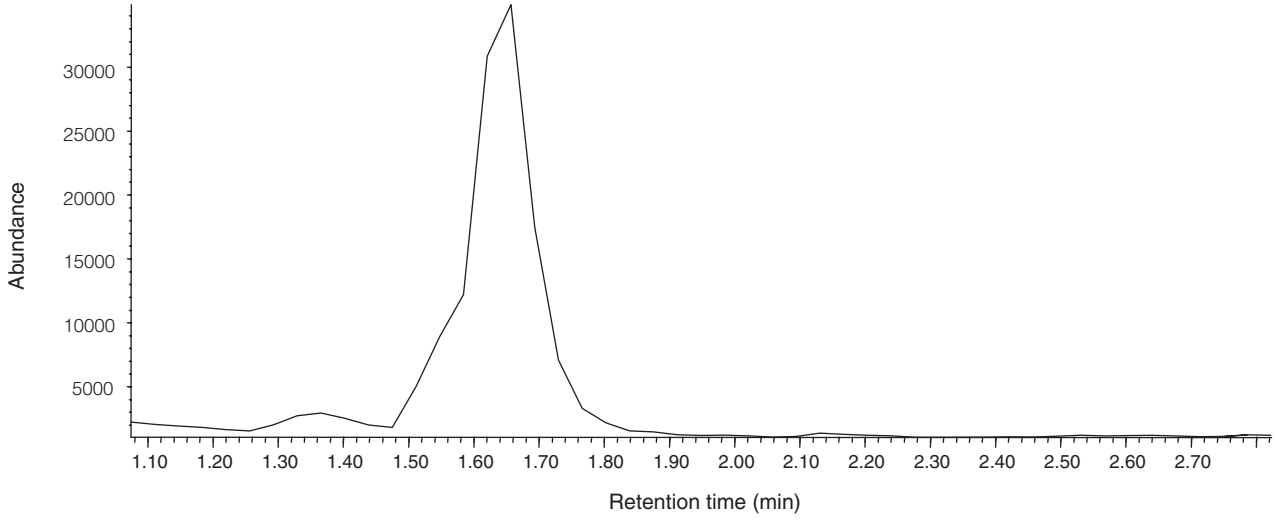

Figure 1. Total ion chromatogram of cutoff calibrator with GC-MS screening method (cutoff concentration of K, NK and DHNK is 100 ng/mL).

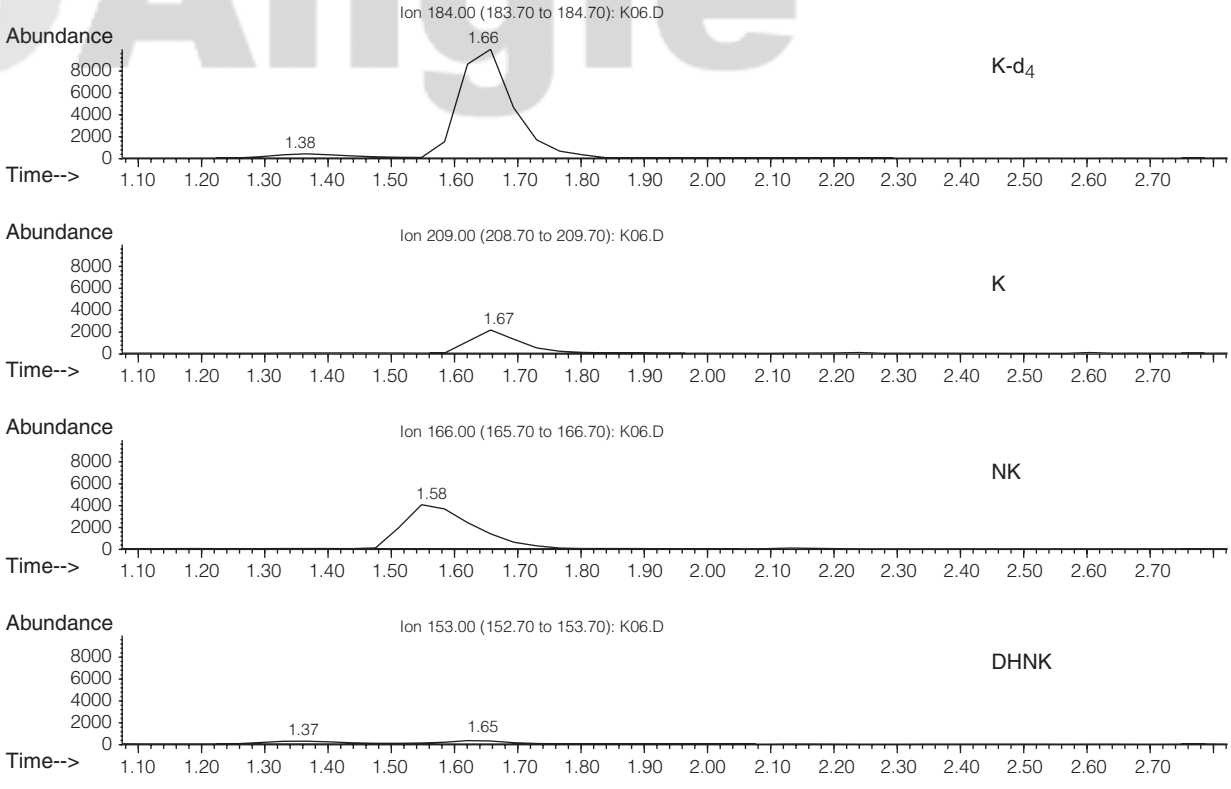

Figure 2. The extract ion chromatogram of cutoff calibrator (K-d_4: *m/z* 184, K: *m/z* 209, NK: *m/z* 166, and DHNK: *m/z* 153).

Table 1. Within-run precision and accuracy of ketamine, norketamine and dehydronorktamine determined with GC-MS screening procedure[a]

		Concentration		
		80 ng/mL	160 ng/mL	400 ng/mL
Ketamine				
	Precision (CV, %)	3.59	1.78	2.34
	Accuracy (error, %)	3.75	2.50	5.75
Norketamine				
	Precision (CV, %)	2.46	1.20	2.46
	Accuracy (error, %)	-5.00	-3.12	-0.75
Dehydronorketamine				
	Precision (CV, %)	8.17	1.41	2.84
	Accuracy (error, %)	-12.50	-11.25	-7.50

[a]Ions monitored: K, *m/z* 209/184; NK, *m/z* 166/184; DHNK, *m/z* 153/184.

Table 2. Within-run and between-day precision and accuracy of dehydronorktamine determined with GC-MS confirmation procedure[a]

		Concentration		
		80 ng/mL	160 ng/mL	400 ng/mL
Within-run				
	Precision (CV, %)	7.90	4.56	9.31
	Accuracy (error, %)	-3.75	-6.67	-9.25
Between-day				
	Precision (CV, %)	2.13	1.58	2.34
	Accuracy (error, %)	-1.56	-1.13	-1.13

[a]Ions monitored: DHNK, *m/z* 153/170.

0.9987. The limit of detection, LOD, (defined as the lowest concentration at which all replicates produce results with signal to noise ratio greater than 3) is 10 ng/mL for K, 10 ng/mL for NK, and 30 ng/mL for DHNK.

II. *Performance Characteristics of the GC/MS Confirmation Procedure*

(I) *Precision and accuracy*

Detailed results were reported in our previous publication[13] except for DHNK. Calibration curves were generated with different concentration of K, NK and DHNK (0, 100, 200, and 500 ng/mL). Linear regression of the calibration curve for DHNK was y = 0.0019x – 0.0108, R^2 = 0.9984. The within-run and between-day precision (%CV) and accuracy (error, %) of three different levels of DHNK at 80, 160, 400 ng/mL are between –9.25% and 9.31%. These data were shown in Table 2.

(II) *Linearity and detection limit*

LOD was 1 ng/mL for K, 5 ng/mL for NK[13] and 20 ng/mL for DHNK. LOQ (defined as the lowest concentration detected that passed the qualifying ion ratio requirement and the concentration determined was within 20% of expected value) was 5 ng/mL for K, 10 ng/mL for NK[13] and 40 ng/mL for DHNK. Range of linearity was 7 μg/mL, 4 μg/mL, and 5 μg/mL for K, NK[13], and DHNK, respectively.

III. *Sensitivity and Specificity*

In order to determine the sensitivity and specificity of GC-MS screening procedure, a group of 163 urine samples collected from disco-dancing club participants were screened with the GC-MS screening method. Every sample was also confirmed with GC-MS. Results are shown in Table 3. There were 88 positive, 74 negative samples and one false negative sample. The screening results of this one false negative sample is 98, 20, 19 ng/mL for K, NK and DHNK, respectively. And the confirmation results of this sample is 103, 37, 79 ng/mL for K, NK and DHNK, respectively. Sensitivity (defined as the number of true positive samples divided by the sum of true positive plus false negative samples) of the GC-MS screening procedure is 98.9% and specificity (defined as the number of true negative samples divided by the sum of true negative plus false positive samples) is 100%.

Table 3. Correlation of the GC-MS screening and GC-MS confirmation method[a]

	GC-MS screening	
GC-MS confirmation	+	–
+	88	1
–	0	74

[a]Cutoff concentration for GC-MS screening and GC-MS confirmation is 100 ng/mL of K, NK or DHNK. Sensitivity: 98.9%, specificity: 100%.

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 with high specificity and sensitivity are the methods of choice to screen drugs of abuse in urine samples[14]. However, there was no commercially available immunoassay for ketamine or its metabolites.

Ketamine is believed to be metabolized to at least two major metabolites: NK and DHNK. Although DHNK was argued to be an artifact[12,15], many reports[7,13] including our data provided evidence to support DHNK as a true metabolite of ketamine. Moore *et al.*[7] employing LC-MS to analyze samples reported that the concentration of DHNK was greater than K and NK concentration in 28 of 33 cases. In this study, the concentration of DHNK was greater than K or NK in 76 of 88 positive cases. There were 18 samples that DHNK is the only analyte with concentration higher than cutoff value (100 ng/mL). These data are in agreement with those reported previously and suggested that DHNK would be the target analyte of choice for screening.

Traditionally, GC equipped with NPD[16], FID[17] and MS[18] were employed to analyze samples with prior extraction, concentration, and derivatization of analytes from biological samples. It may take one to two hours in some method[16,18]. The long analytical time rendered those methods not suitable for screening purpose.

In this study, we presented a simple screening procedure for K, NK, and DHNK. There was just one liquid-liquid extraction step without further evaporation to dryness. The whole extraction procedure took less than 10 min and used less organic solvent (200 μL of hexane). An experienced technician can process 60 samples in one hour. The total analytical time is 5 min, including temperature equilibrium. No solvent blank was run in between samples to speed up the process. It's because any false positive results will be corrected by subsequent confirmation procedure. The analysis time of GC/MS confirmation method is 20 min/sample. A blank was usually added between samples during analysis to prevent carryover. The analysis time of confirmation method is thus 40 min/sample. Compared with the analysis time of screening method (5 min/sample), the screening procedure required only 1/8 of the instrument analysis time. Cutoff concentration for GC-MS screening and GC-MS confirmation was set at 100 ng/mL of K, NK and DHNK. Good correlation between GC-MS screening and confirmation was obtained (sensitivity is 98.9% and specificity is 100%).

Although the LOD of our method was less than 10 ng/mL, the cutoff was set at 100 ng/mL, substantially higher than the LOD. This is in accordance with the guideline established by the Department of Health, Taiwan[14]. For example, the LOD for morphine and codeine was 30 ng/mL and 10 ng/mL, respectively, in one study[19]. The cutoff concentration for morphine and codeine in GC/MS confirmation assay was set at 300 ng/mL[14].

It is a common practice to employ methods with different analytical principles for screening and confirmation purposes. However, when there were discrepancies between the screening and confirmation results, GC/MS

confirmation results was the one accepted (gold standard). Theoretically only the GC/MS confirmation was required to obtain results. The main purpose of a screening procedure was to screen out negative samples before confirmation in order to save time and money. Our screening procedure satisfied the two purposes of a screening program: saving time (requires only 1/8 of the time) and money (in terms of instrument time). Presumptive positive samples were confirmed with GC/MS procedure. As other screening procedure, ours was capable of screening out negative samples before confirmation. We have analyzed 163 samples and observed no false positive result.

The GC-MS screening method we described is rapid, sensitive and specific. It can be employed to screen K, NK and DHNK in urine samples.

CONCLUSIONS

The screening procedure is the first reported procedure to simultaneously analyze K, NK and DHNK. The GC-MS screening method is rapid, sensitive and suitable for high volume screening for ketamine abuse.

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