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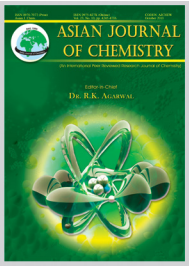

Determination of Ketamine in Human Urine by Gas Chromatography

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An analytical method using microwave assisted extraction-gas chromatography is developed for the determination of ketamine in human urine. For the detection of ketamine, urine samples are extracted on an microwave assisted extraction apparatus. Extracted calibration curve is linear from 0.5 mg/L to 100 mg/L for ketamine with correlation coefficients exceeding 0.999. The limit of detection is 0.2 mg/L for ketamine. The limit of quantitation is 0.5 mg/L for ketamine. The recoveries of ketamine are from 79.50 to 101.31 %. The intraday and interday run precisions (CV) for ketamine are less than 2.84 %. The analytical method is applied to five suspected urine samples and the concentration of ketamine is determined. Ketamine and its metabolite (norketamine) are confirmed by GC-MS. Microwave assisted extraction is simple and needs less organic solvent than liquid-liquid extraction and solid-phase extraction and gas chromatography can offer both qualitative and quantitative information for urinalysis of ketamine in forensic analysis.

Key Words: Microwave-assisted extraction, Gas chromatography, Ketamine, Urine.

INTRODUCTION

Ketamine is a dissociative anesthetic agent that has been widely used in clinical practice. On the other hand, it is widely abused for hallucination and also misused as a "date-rape" drug in recent years. Developing a rapid, sensitive and reliable analytical method to determine the content of ketamine in human body fluid is very important for the clinical and forensic science. Published methods for the determination of ketamine in urine or blood samples were mainly high performance liquid chromatography (HPLC)[1-6] or liquid chromatography-mass spectrometry (LC-MS)[7-14], ultra performance liquid chromatography (UPLC)[15], micellar electrokinetic chromatography (MEKC)[16-18] and gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS)[19-30]. Ketamine and its metabolites are usually extracted from biological samples, such as human hair[18,23-25], urine[1,8,10-12,17,19,21,22,26-28], blood[2-6,13,14,29] and tissue[30] by liquid-liquid extraction (LLE)[2,3,9,20,21,28], solid-phase microextraction (SPME)[1] and solid phase extraction (SPE)[4,7,8,11-16,22-24,26,27]. Microwave-assisted extraction (MAE) has recently attracted increasing interest because it enables rapid extraction with efficiency comparable with that of classical techniques, such as LLE. Microwave-assisted extraction has been widely used for the preparation of different samples, such as blood[31], urine[32-35] and tissues[36].

Based on previous report[33], the goal of this paper is the estimation of microwave-assisted extraction-gas chromatography method for the determination of ketamine in human urine. Present results indicated that microwave-assisted extraction coupled with gas chromatography analysis could be used for the determination of ketamine in human urine.

EXPERIMENTAL

Ketamine hydrochloride was purchased from National Laboratory of Narcotic Drug (Beijing, China). SKF525A was used as internal standard. Methanol (HPLC grade) was bought from Sigma (St. Louis, MO, USA). Sodium carbonate, sodium bicarbonate, ethyl acetate, cyclohexane, chloroform and isopropanol were of analytical reagent grade and were obtained from Beijing Chemical Company (Beijing, China). Purified water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Drug-free urine samples were obtained from non-drug users and urine specimens were from suspected drug users submitted by police. All urine samples were stored at -80 ºC until analysis.

Microwave-assisted extraction procedure: Microwave-assisted extraction was performed in a microwave extractor (MSP-100E, Beijing Leiming. China), equipped with pressurized vessels. 1 mL of spiked urine samples (containing 60 µL 100 mg/L ketamine and SKF525A) was transferred into a PTFE vessel and then the pH values of samples were adjusted by NaOH solution (4 mol/L). 4 mL of extraction solvent was added, with vessels tightly closed and placed into the micro-

wave sample preparation system. The extraction process was carried out at 50 ºC for 8 min with a constant power of microwave (85 W). After extraction, the vessels were left in the extraction system to cool down to room temperature. The content of all vessels were transferred to labeled glass tubes and then centrifuged for 10 min (10,000 rpm). After that the upper layer was successively transferred to Eppendorf vials and evaporated under nitrogen stream at room temperature to dry residues. Dry residues were dissolved in 100 µL of methanol and vortexed for 2 min and 1 µL of result solution was injected for GC analysis.

Gas chromatography analysis: Gas chromatography analysis was carried out with a 6890N series gas chromatography equipped with flame-ionization detection (FID) and with a cryogenic oven temperature device (Agilent Technologies, Palo Alto, CA, USA). A HP-5 capillary column (30 m × 0.32 mm i.d., 0.5 µm thickness of crosslinked 5 % phenyl methyl silicone) was used for separation. The column oven was initially held at 170 ºC for 2 min, the temperature was programmed to 250 ºC at a rate of 10 ºC/min and then the temperature was raised to 280 ºC at a rate of 30 ºC/min; this temperature was maintained for 8 min. The injection port temperature was 280 ºC and the FID temperature was 300 ºC. Nitrogen was used as carrier gas at a flow rate of 3.0 mL/min.

GC-MS confirmation: An Agilent 6890 GC/5973 MSD system was used in this study using a HP-5 MS column (30 m × 0.25 mm I.D., 0.25 µm film thickness). GC oven temperature was programmed to rise initially from 130 to 170 ºC at 10 ºC/min, to 200 ºC at 5 ºC/min and then to 280 ºC at 30 ºC/min and finally held at this temperature for 0.5 min. A 2-µL aliquot was injected in splitless mode. The injection port and the transfer line temperature were set at 280 ºC and 300 ºC, respectively. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The MS was operated in electronic impact mode and selected ion monitoring (SIM) mode was used for the identification of ketamine and norketamine. The five ions of each target compound were chosen for the evaluation under SIM mode, m/z 138, m/z 152, m/z 180, m/z 182 and m/z 209 for ketamine and m/z 131, m/z 149, m/z 166, m/z 168 and m/z 195 for norketamine.

RESULTS AND DISCUSSION

Optimization of microwave-assisted extraction parameters: In order to improve the extraction efficiency of ketamine in urine sample, different parameters in microwave-assisted extraction experiments were investigated, such as extractant and its amount, pH value of sample, extraction time and temperature.

The effects of different extractants including ethyl acetate, cyclohexane, chloroform-isopropanol (3:1, v/v) were studied. The results showed that the high extraction yields were obtained by ethyl acetate and ethyl acetate was selected as extractant for ketamine in urine samples. And then the volume of ethyl acetate used in extraction procedure was investigated; the optimal volume of extractant was 4 mL (Fig. 1). To evaluate the effect of extraction temperature on the extraction seven temperature levels were tested: 30, 40, 50, 60, 70, 80 and 90 ºC. Fig. 2 showed the temperature of extraction was set as 50 ºC. The effect of microwave time on microwave-assisted extraction yield of ketamine was investigated at 85 W when 4 mL ethyl acetate was used as extractant. The extraction time was investigated for 4, 6, 8, 10, 12 and 14 min, respectively. Upon raising the extraction time of microwave from 4 to 14 min, the extraction rate of all analytes increased to the maximum values at the time of 8 min and decreased gradually when the extraction time was more than 10 min (Fig. 3), so the extraction time of microwave-assisted extraction was set as 8 min for further experiments. The effect of pH value of urine sample on microwave-assisted extraction extraction was studied. Fig. 4 showed that the extraction rate increased with the increasing of pH in the range of pH 10-12 and the extraction rate was decreased when urine sample with pH 12.5 was tested, so the pH value of urine samples were adjusted to pH 12 prior to microwave-assisted extraction procedure.

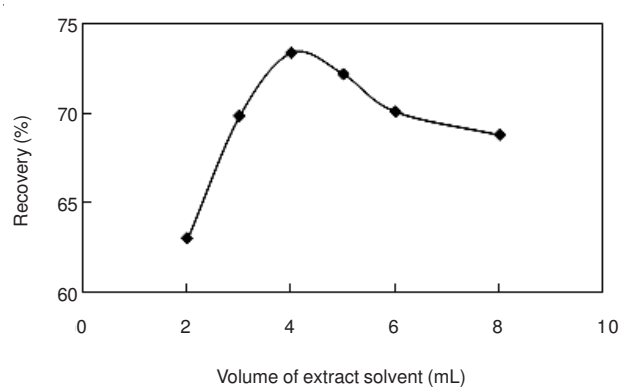

Fig. 1. Effect of extractant volume on extraction rate of ketamine from urine. Microwave assisted extraction conditions: ethyl acetate was used as extractant. The pH values of urine or spiked urine samples were pH12; extraction was performed at 40 ºC for 10 min with a constant power of microwave (85 W)

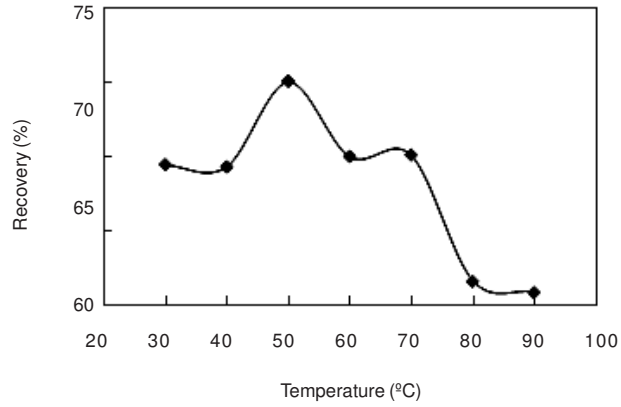

Fig. 2. Effect of extraction temperature on extraction rate of ketamine from urine. Microwave assisted extraction conditions: 4 mL of ethyl acetate was used as extractant, the range of extraction temperature was from 30 ºC to 90 ºC and other conditions as Fig. 1

As a result of optimization, the microwave-assisted extraction experiments were carried out with the optimal conditions: 4 mL ethyl acetate was used as extractant. The pH values of urine or spiked urine samples were pH 12; microwave-assisted extraction was performed at 50 ºC for 8 min with a constant power of microwave (85 W). Fig. 5 showed the typical chromatogram of spiked urine sample.

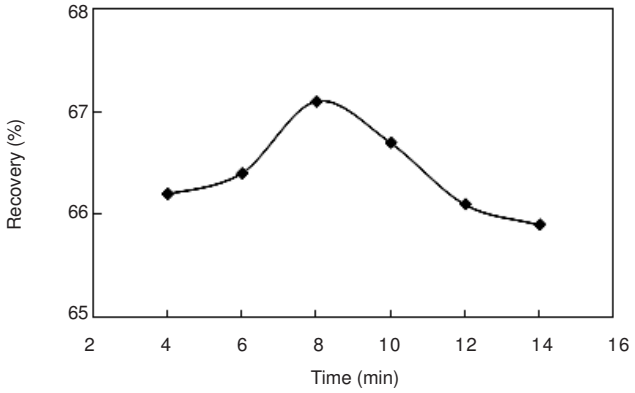

Fig. 3. Effect of extraction time on extraction rate of ketamine from urine. Microwave assisted extraction conditions: the range of extraction time was from 4 min to 14 min; and other conditions as Fig. 1

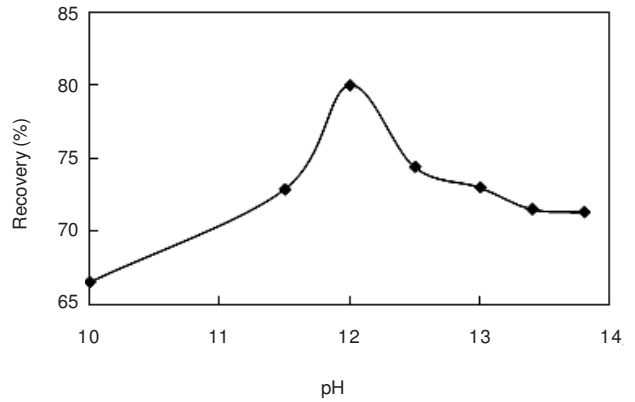

Fig. 4. Effect of sample pH on extraction rate of ketamine from urine. Microwave assisted extraction conditions: the range of sample pH was from pH10 to pH13; and other conditions as Fig. 1

Confirmation of Ketamine and its metabolite: The extracts of suspected specimens by microwave-assisted extraction were analyzed by GC-FID. The typical chromatogram of GC-FID was shown in Fig. 6. According to the retention time of ketamine and internal standard in Fig. 5, these target compounds were identified in Fig. 6. For future confirmation the extracts were analyzed by GC-MS method and mass spectrum of ketamine was shown in Fig. 5, some characteristic ions of ketamine were determined, including m/z 138, m/z 152, m/z 180, m/z 182 and m/z 209.

Except for the ketamine and internal standard, other peaks in total ion chromatogram were scanned for the confirmation of the metabolite of ketamine. According to the mass spectrum, some characteristic ions of norketamine were detected in the full scan mass spectrum of unknown peak with retention time of 7.09 (Fig. 6), so the peak was identified as norketamine (NK), a main metabolite of ketamine.

Evaluation of developed method: The calibration plot for ketamine was linear over the concentration range of 0.5 mg/L-100 mg/L by GC analysis; regression equation of ketamine (y = 0.0171x-0.0125, r = 0.9994) was obtained by plotting the rate (y) of peak-area between ketamine and internal standard *versus* the analyte concentration (x) in spiked urine samples. The regression equations described above were used to calculate concentration of ketamine in urine samples.

The limit of detection was defined as the lowest concentration of analytes spiked in urine that could be detected with a signal-to-noise ratio (S/N) of at least 3. The detection limits of ketamine by GC-FID detection was 0.2 mg/L. The LOQ of the developed method for ketamine was 0.5 mg/L.

The real case samples and their spiked urine samples with ketamine standard solution were analyzed by the developed

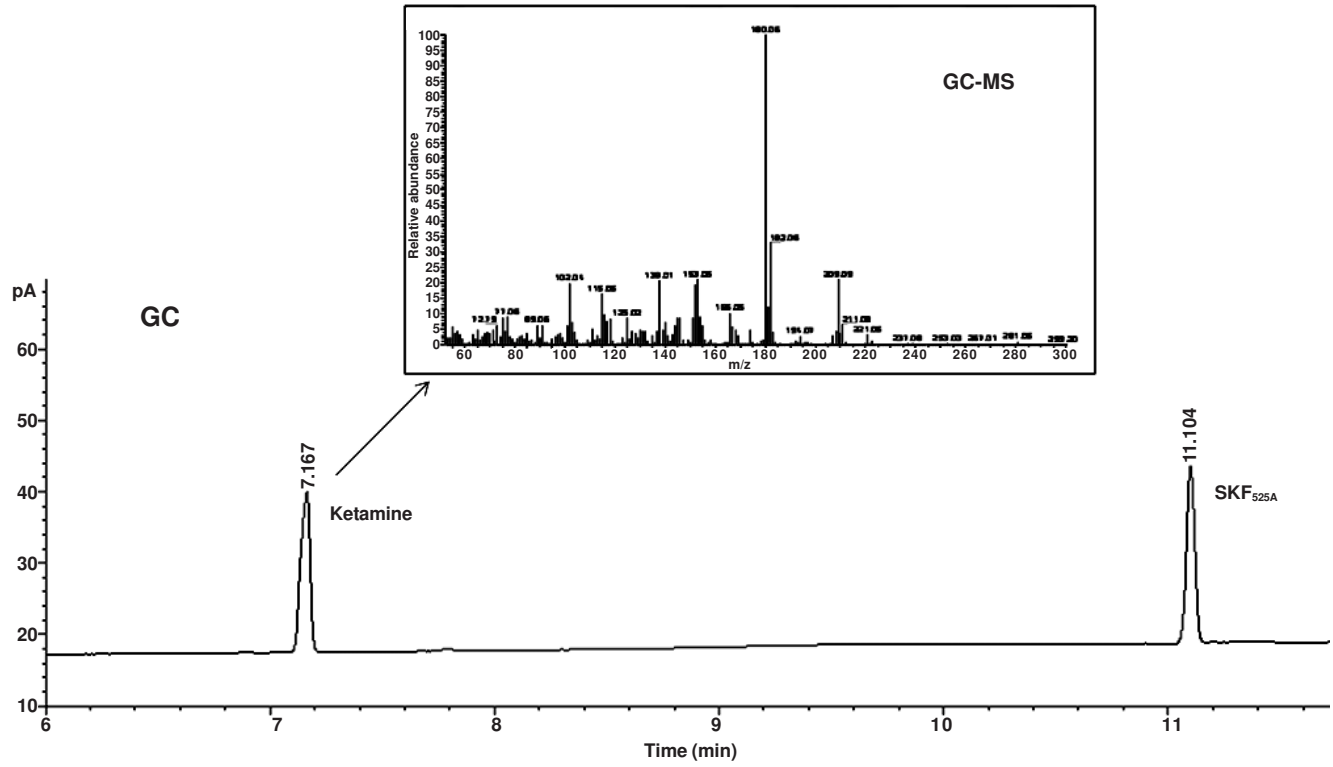

Fig. 5. Typical chromatograms of spiked urine sample. MAE procedure was carried out under the optimal parameters and ketamine were identified by GC-MS method

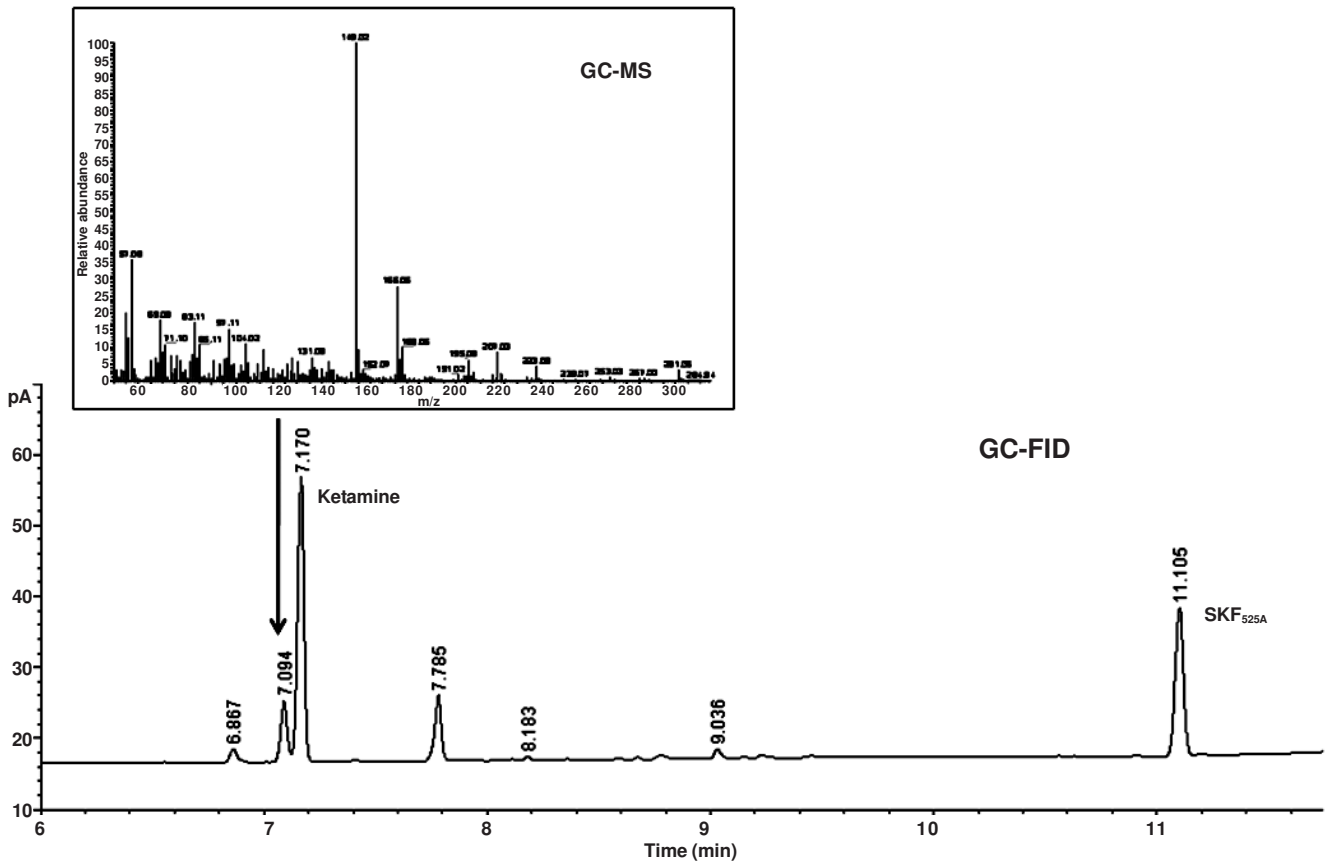

Fig. 6. Typical chromatograms of real urine samples from forensic cases by GC-FID. Microwave assisted extraction procedure was carried out under the optimal parameters and the metabolite of ketamine were identified as norketamine by GC-MS

method and the recovery rate were calculated. Our results indicated that the recovery of microwave-assisted extraction method was in the range of 79.50 %-101.31 % (n = 5). The intra and interday relative standard deviations (RSD) for ketamine in urine were 2.20 % and 2.84 % (n = 5), respectively.

Determination of ketamine in urine samples: The concentration of ketamine in suspected urines were in the range of 2.71-11.53 mg/L (Table-1) and the chromatogram of real urine sample (Fig. 6).

TABLE-1
CONTENT OF KETAMINE IN REAL URINE SAMPLES BY MAE-GC

No. of samples	Measured value (mg/L)	RSD (%) (*n*=3)
1	8.61	0.87
2	2.71	1.77
3	11.53	1.64
4	11.05	1.23
5	5.66	1.89

Conclusion

An efficient, quick and reproducible method was developed for the determination of ketamine in human urine by using microwave-assisted extraction and GC analysis. Our primary results indicated that the microwave-assisted extraction method was found to be simple, easy to perform and inexpensive and could be used for the preparation of urine samples for GC and other chromatographic techniques.

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