

# **Determination of Ketamine and Metabolites in Urine by Liquid Chromotography-Mass Spectrometry**

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## **Abstract**

Ketamine (2-chlorophenyl)-2-(methylaminocyclohexanone) is commercially available as a racemate and mainly used as a short-acting anesthetic agent. Ketamine is also one of the most potent hallucinogens known. It is easily found on illicit market, PUB and become a severe problem in the modern society. Suspect's urine is a necessary sample in the forensic identification. The pretreatment procedures of urine samples usually are complicated and time consuming. A simple and effective technique for urine sample analysis becomes necessary. This project will investigate the feasibility if applying LC/MS and LC/MS/MS for the determination of ketamine and its metabolites in the urine samples. In this study, we systematically evaluated the optimum procedures including atmospheric pressure ionization and electrospray ionization modes of mass spectrometry for analyzing ketamine and its metabolites. To demonstrate the proposed method's applicability, the detection limits, linear dynamic detection ranges and reproducibility are studied by determining the amount of ketamine. The metabolic compounds of ketamine in urine will also be studied.

The chromatographic system consisted of Surveyor MS Pump and detector is Thermal Finnigan LCQ. Chromatographic separation was achieved on a SUPELCOSIL LC-18 column (4.5×250mm, 5 μ m) with an isocratic mobile phase of acetonitrile-0.03M ammonium acetate buffer (50:50 vol) adjusted to pH 7.1. In mass spectrometer, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are used for analyzing ketamine, norketamine and dehydronorketamine.

Ketamine and its two major metabolites norketamine and dehydronorketamine analyzed by LC/MS ESI positive mode were optimized with spray voltage for 6.5kV, capillary temperature for 160 °C, sheath gas flow rate and auxiliary gas flow rate are 1.35L/min and 6 L/min respectively. When target compounds are analyzed by APCI positive mode, vaporizer temperature for 250 °C, capillary temperature for 160 °C, discharge current for 3μA, sheath gas flow rate and auxiliary gas flow rate for 1.35L/min and 6 L/min respectively are the optimal conditions.

## **Introduction**

Ketamine (2-chlorophenyl)-2-(methylaminocyclohexanone) is commercially available as a racemate and mainly used as a short-acting anesthetic agent. It was reported that ketamine produced analgesic effects for neuropathic pain of tolerance to antinociceptive effects of morphine when administered in a low dose without anesthetic. Ketamine is also one of the most potent hallucinogens known. It is easily found on illicit market, PUB and become a severe problem in the modern society. Suspect's urine is a necessary sample in the forensic identification. The pretreatment procedures of urine samples usually are complicated and time consuming. A simple and effective technique for urine sample analysis becomes necessary.

This project will investigate the feasibility of applying LC/MS and LC/MS/MS for the determination of ketamine and its metabolites in the urine samples. In this study, we systematically evaluated the optimum procedures including atmospheric pressure chemical ionization and electrospray ionization modes of mass spectrometry for analyzing ketamine and its metabolites. To demonstrate the proposed method's applicability, the detection limits, linear dynamic detection ranges and reproducibility are studied by determining the amount of ketamine. The metabolic compounds of ketamine in urine will also be studied.

## **Experiment**

### **Standards**

Ketamine and norketamine were purchased from Cerilliant (Cerilliant, U.S.A.), dehydronorketamine was obtained from Pfizer (Pfizer, U.S.A.).

### **HPLC condition**

HPLC analysis were carried out on a Surveyor MS pump (Thermal Quest, U.S.A.). Chromatographic separation was performed on a Supelcosil<sup>TM</sup> LC-18DB column (5  $\mu$  m particle size ) 4.6 $\times$  250 mm with precolumn filter of 3 mm frit. The mobile phase consisted of acetonitrile:0.03M ammonium acetate buffer(50:50 by vol) adjusted to pH 7.1. The flow rate of the mobile phase was 1.0 mL/min.

### **MS condition**

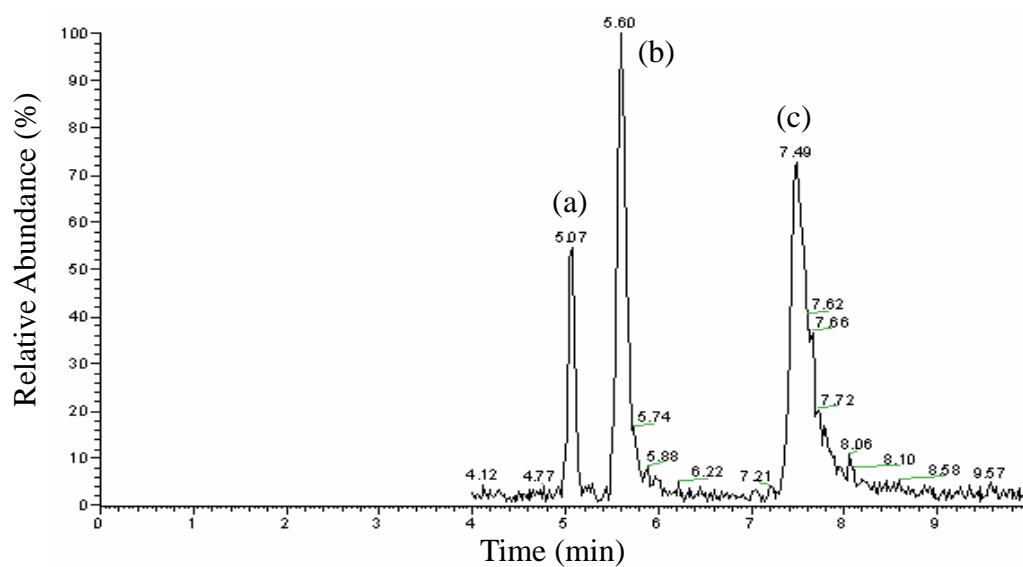
A Finnigan LCQ quadrupole ion trap mass spectrometer was used in positive ionization mode. Full scan spectra were taken in the mass range  $m/z$  150~330. The capillary temperature, vaporizer temperature, sheath gas and auxiliary gas flow rate, discharge current and spray voltage were all optimized with regard to maximum signal intensity of protonated

molecule by flow injection of 5-  $\mu$  L samples (0.5mg/L).

### Sample preparation

All urine samples were filtered through a 0.2  $\mu$  m nylon syringe filter (Millipore) then direct injected into the column.

### Result and Discussions



Mass ion chromatogram of spiked 20 ng/mL standards (a) dehydronorketamine (b)norketamine (c)ketamine in urine produced by HPLC/(+) APCI/MS

Table 1. The optimum ESI MS parameters for determination of ketamine and its two metabolites

MS parameter	Value
Spray Voltage ( kV )	6.5
Capillary Temperature ( )	160
Sheath Gas Flow Rate ( L/min )	1.35
Auxiliary Gas Flow Rate ( L/min )	6
Capillary Voltage ( V )	45
Tube Lens Offset ( V )	-15
Octapole 1 Offset ( V )	-0.25
Lens Voltage ( V )	-15
Octapole 2 Offset ( V )	-5.5
Octapole RF Amplitude ( V p-p )	400

Table 2. The optimum APCI MS parameters for determination of ketamine and its two metabolites

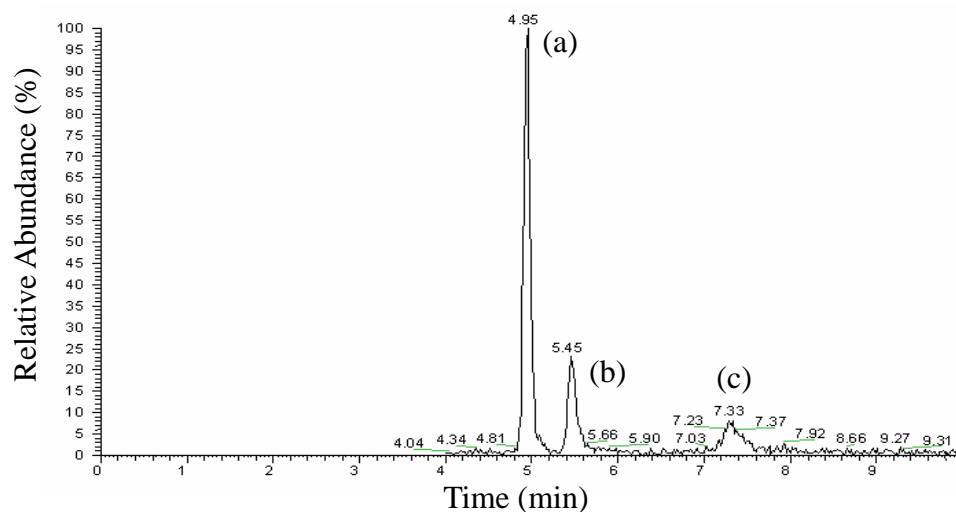
MS parameter	Value
Vaporizer Temperature ( )	250
Capillary Temperature ( )	160
Discharge Current ( $\mu$ A )	3
Sheath Gas Flow Rate ( L/min )	0.75
Auxiliary Gas Flow Rate ( L/min )	0
Capillary Voltage ( V )	11
Tube Lens Offset ( V )	-25
Octapole 1 Offset ( V )	-1.25
Lens Voltage ( V )	-25
Octapole 2 Offset ( V )	-7.5
Octapole RF Amplitude ( V p-p )	400

Table 3. Estimated linear range, correlation coefficient, LOD and precision for LC-APCI/MS for ketamine and its metabolites in urine

Compound	Linear range (ng/L)	Correlation coefficient (R <sup>2</sup> )	LOD*	R.S.D.
Ketamine	12.5-200	0.9991	0.93	4.4
Norketamine	12.5-200	0.9998	0.02	3.3
Dehydronorketamine	12.5-200	0.9997	0.02	4.5

\* S/N = 3

n = 6, concentration 20 ng/L



Mass ion chromatogram of patient urine doped 10mg ketamine produced by HPLC/(+)APCI/MS

## Conclusion

In this work, LC-APCI/MS to evaluated for trace level determination of ketamine, norketamine and dehydronorketamine in urine. Chromatographic separation was achieved on a SUPELCOSIL LC-18 column (4.5×250mm, 5 μ m) with an isocratic mobile phase of acetonitrile-0.03M ammonium acetate buffer (50:50 vol) adjusted to pH 7.1. When target compounds are analyzed by APCI positive mode, vaporizer temperature for 250 °C, capillary temperature for 160 °C, discharge current for 3μA, sheathe gas flow rate and auxiliary gas flow

rate for 1.35 L/min and 6 L/min respectively are the optimal conditions. The linearity was obtained with a precision below 5 % R.S.D. and linear range from 12.5~200 ng/L. Detection limit were estimated at the low ng/L levels, for ketamine, 0.93 ng/L, for both norketamine and dehydronorketamine, 0.02 ng/L. We succeed in application of the analytical methods to the determination the concentration of ketamine and its two metabolites in patient's urine. The target compounds were found in concentration ranging are 1~131 ng/L for ketamine, 0.2~131 ng/L for norketamine, 1~312 ng/L for dehydronorketamine.