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Detecting ketamine in beverage residues: Application in date rape detection

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Ketamine can be used to facilitate date-rape when unknowingly spiked into a victim's beverage. If a biological sample is not available from the victim, the beverage container might be the only remaining source of forensic evidence. We present a rapid, simple analysis method for the detection of ketamine in wet or dry beverage residues based on liquid chromatography-mass spectrometry (LC-MS). Wet residues consist of the final few drops (<1 ml) in a container while dry residues are the remains once all liquid has evaporated. By using LC-MS, which readily handles aqueous samples, often no derivatization or sample extraction is needed, thus reducing analysis time and lab technician involvement. Tandem mass spectrometry (MS/MS) provides an enhancement in both selectivity and sensitivity. We have studied a range of beverages and determined limits of detection between 1.2×10–3 and 1.3×10–4 mg/ml, compared to 0.21–0.85 mg/ml used in most date-rape scenarios. This paper represents the first published report of using LC-MS/MS for the analysis of beverage residues for the presence of a date-rape drug. This method could replace the current gas chromatography-mass spectrometry (GC-MS) methods and provide a faster, more selective method for the analysis of date-rape drugs, requiring virtually no sample preparation. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: ketamine; date-rape; LC-MS; residue; beverage

Introduction

Drug-assisted rape is a serious issue, with a recent report estimating that 5% of the entire female population, and 6.4% of women in college, have been the victim of drug- or alcohol-facilitated rape.[1] Ketamine, a synthetic anesthetic[2] originally created as an alternative to phencyclidine (PCP),[3] induces psychedelic effects, similar to those experienced with PCP, such as floating sensations, dream-like hallucinations, and increased arousal,[3] and can also lead to a cataleptic state with accompanying amnesia.[4,5] Also known as K, Special K, Vitamin K, Ket, Purple, and Green, it has been found to be popular at rave scenes.[6,7] These properties make it an attractive drug for the facilitation of date-rape. As with most date-rape drugs, ketamine-induced amnesia may prevent the victim from realizing a rape has occurred until a significant amount of time has passed. Additionally, rape victims often do not come forward initially. Both of these issues can result in an inability to collect biological evidence such as blood or urine samples. Ketamine clears body fluids (blood and urine) in approximately 48 h,[8,9] so the victim must come forward within this time period if biological evidence of the crime is to be collected.

Numerous methods have been described for the analysis of ketamine in various matrices. Urine can be analyzed for the presence of ketamine using gas chromatography–mass spectrometry (GC-MS) by either derivatizing ketamine prior to analysis[10] or by first employing a liquid-liquid extraction.[11] GC-MS has also been used to detect ketamine in hair of recreational drug users.[12] In order to eliminate the need for time-consuming derivatization or extraction steps, we decided against using GC-MS as the detection method. High performance liquid chromatography (HPLC) has been used to analyze for ketamine in urine.[13] This method utilized solid-phase microextraction (SPME) prior to HPLC analysis. HPLC has also been used to analyze ketamine in blood plasma.[14] The lack of mass spectral analysis in these methods does not allow the unambiguous identification of ketamine, as other species may have similar retention times. Liquid chromatography–mass spectrometry (LC-MS) has been used to analyze ketamine in serum[15,16] and urine.[17] LC-MS, while more specific than LC-only methods, often does not unambiguously identify the drug, since most LC-MS methods utilize electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI), both of which produce primarily intact protonated molecules, with little compound-specific fragmentation observed. Thus, while analytical methods exist for the detection of ketamine in a variety of matrixes, these methods often require extensive sample preparation (GC-MS), or do not provide unambiguous identification of ketamine in a sample (LC or LC-MS).

As already described, biological samples require sample collection shortly after the administration of the date-rape drug, which may not always be possible. In order to provide a non-biological means of determining the presence of ketamine in a potential date-rape scenario, we aimed to develop a method for the analysis of ketamine in various beverage residues. A GC-MS method for the analysis of gamma-hydroxybutyric acid (GHB) in liquid beverage (Coca-Cola®, beer, and lemonade) samples has been described.[18] This method employed SPME and chemical derivatization before analysis. The total sample preparation time (including extraction and derivatization) was over 1 h per sample. The goal of the work presented here was to provide a fast, simple, sensitive, and specific method of analysis. In order to make the method as useful as possible, we aimed to reduce as much

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prior-sample preparation as possible as this is usually a time-consuming, and thus an expensive process.

ESI requires a liquid sample, eliminating the need for prior extraction or chemical derivatization as is often used in GC-MS methods. Tandem mass spectrometry (MS/MS) can provide ion fragments specific to the chosen analyte ion. Since MS/MS requires the selection of a precursor ion, and removal of all other non-selected ions, the amount of chemical noise is automatically reduced. This can lead to lower limits-of-detection. Since date-rape drugs are usually surreptitiously slipped into liquid beverages, LC-MS/MS may represent an ideal platform for the analysis of date-rape drugs, such as ketamine, in beverages, and is the focus of this study. A previous LC-MS/MS analysis of drugs of abuse, including ketamine, in wastewater has been published using ultra performance LC (UPLC).[19] LC-MS/MS has also been used to analyze for ketamine in urine samples.[20]

For this study, we chose four different beverages to analyze: tap water, Coca-Cola®, beer, and a fruit smoothie. These were chosen to represent the range of possible beverages that would commonly be consumed in a date-rape scenario. Beer was chosen to represent alcoholic beverages and the fruit smoothie was used to represent all blended fruit beverages including daiquiris and other fruity alcoholic beverages. Both wet and dry samples were collected. The latter sample type represents the potential evidence that would remain in the glass after beverage consumption up until the container is washed. This residue may be the only chemical evidence remaining if sufficient time has passed to eliminate all biological evidence. The ability to determine precisely which beverage container contained the spiked beverage may also be useful in identifying the person responsible for spiking the beverage.

Methods

Solvents and materials

HPLC grade methanol was purchased from J.T. Baker (Phillipsburg, NJ, USA) and formic acid was purchased from Acros Organics (Morris Plains, NJ, USA). Pure, 18 MΩ-cm, water was obtained using a Barnstead Nanopure Series 550 filtration system (Dubuque, IA, USA). Ketamine hydrochloride was purchased from Sigma Aldrich (St Louis, MO, USA). Deuterated standard (100 μg/ml in methanol), ketamine-d_4 hydrochloride, was purchased from Cerilliant (Round Rock, TX, USA). Coca-Cola® soda and Leinenkugel's® ale (beer) were purchased at a local supermarket. Fruit smoothies were purchased at the campus café and were made from crushed ice, orange juice, blueberries, strawberries, and raspberries.

Preparation of standard stock solutions

For each of the four beverages studied – water, Coca-Cola® (soda), Leinenkugel's® Original Ale (beer), and fruit smoothie – ketamine hydrochloride was accurately weighed and diluted with the beverage to a concentration of 1.5 nmol/μl (0.36 mg/ml) to make an initial stock solution. Working solutions were diluted from the stock solution, using the beverage being studied, to yield concentrations of 0.0025, 0.005, 0.05, 0.5, 1.0, and 1.5 nmol/μl (0.00059, 0.0012, 0.012, 0.12, 0.24, 0.36 mg/ml, respectively). Deuterated ketamine was added to each solution at a concentration of 5 μg/ml as an internal standard.

Preparation of spiked beverage samples

For each beverage, two different samples were collected. A wet sample was collected from the final few drops that remain at the bottom of the beverage container after consumption. A dry sample was collected from the dried residue that remains at the bottom of the container after the final few drops have been allowed to sit overnight and the liquid has evaporated. To simulate a date-rape scenario, 250 ml of spiked beverage working solution was poured into a drinking glass. The glass was gently agitated every 30 s. After 5 min, the spiked solution was poured out of the glass to simulate beverage consumption. For wet sample collection, 5 ml of nanopure water was used to rinse down the sides of the drinking glass immediately after the spiked beverage was removed. A 1 ml aliquot was then removed and stored in a polypropylene vial at 4 °C. For dry sample collection, after pouring out the spiked beverage the drinking glass was left overnight to dry. The following morning, 5 ml of nanopure water was used to rinse down the sides of the glass and dissolve any dried residue. The solution was allowed to sit in the glass for 5 min. A 1 ml aliquot was then removed and stored in a polypropylene vial at 4 °C.

Filtration of smoothie samples

The presence of fruit pulp and seeds in fruit smoothies was included in order to study the effect these have on the analysis. Smoothie samples required additional preparation prior to analysis in order to remove the solid particulate matter. The 1-ml sample aliquot was placed in a microSpin 17 centrifuge unit (Fischer Scientific, Waltham, MA, USA) and spun at 14 000 RPM for 30 s. Following centrifugation, the supernatant was placed in a 10-ml glass syringe that had been fitted with a piece of 740-E analytical filter paper (Schleicher & Schuell, Keene, NH, USA). The supernatant was pushed through the glass syringe and subsequently centrifuged for 60 s at 14,000 RPM. The supernatant was then collected and loaded into a 3-ml syringe. A 0.45 μm Acrodisc syringe filter (Pall Gelman, East Hills, NY, USA) was attached and the sample was pushed through the syringe, collected, and stored for subsequent analysis.

HPLC/MS-MS analysis

Separation was performed using an Agilent 1100 series capillary HPLC unit and a ZORBAX Eclipse XDB-C18 column (50 mm × 1 mm i.d., 3.5 μm particle size, Agilent Techologies, Santa Clara, CA, USA). Using a glass syringe (Hamilton, Reno, NV, USA), 10 μl of sample was injected into a six-port injector containing a 5 μl sample loop. A linear gradient elution program (5% B to 95% B [solvent A: 0.1% (*v/v*) formic acid in water; solvent B: 0.1% (*v/v*) formic acid in MeOH]) was used over 20 min at a column flow rate of 4 μl/min. The HPLC eluate was directly interfaced to the ESI source of an ion-trap mass spectrometer (Esquire 3000+, Bruker Daltonics, Billerica, MA, USA). The capillary voltage was set at −4500 volts. Nitrogen gas was used both for nebulization, at 4.0 l/min, and as the drying gas, at a pressure of 15.0 psi with a dry temperature of 290 °C. Selected reaction monitoring (SRM) was used to increase the signal-to-noise ratio, while also providing increased selectivity. The *m/z* 237.7 ion, representing the intact protonated ketamine molecule $[M+H]^+$, was isolated and helium gas was used to promote fragmentation. Following each analysis, the syringe was washed with five volumes of MeOH followed by five volumes of

water. A 5 μl water injection blank was run following each analysis to quantify the amount of background noise.

Data analysis

After data collection was completed, an extracted ion chromatogram (EIC) of *m/z* 219.9, a characteristic product ion that results from the fragmentation of ketamine, was obtained using Bruker DataAnalysis software. This significantly reduced the chemical background noise, since signal was observed only when an ion of *m/z* 237.7 eluted into the mass spectrometer and was subsequently fragmented into a product ion of *m/z* 219.9. While *m/z* 219.9 was used for quantitation, fragment ions at *m/z* 125, 179, 207, and 219.9 were used to confirm that the compound being analyzed was ketamine. The use of four fragment ions provides a more specific identification of the analyte as ketamine. Each sample was analyzed three times, with the exception of the lowest concentration for each sample type which was analyzed seven times, and the *m/z* 219.9 EIC peak area was calculated for each analysis and each blank using Bruker QuantAnalysis software. The peak area from the preceding blank was subtracted from each sample peak area to correct for background noise. The resulting net peak areas were then averaged for each concentration and sampling method.

Limit-of-detection (LOD) and limit-of-quantitation (LOQ) values for each beverage and sampling method were also calculated using the following definitions:[21]

$$\mathrm{LOD} = \frac{3s}{m} \quad (1)$$

$$\mathrm{LOQ} = \frac{10s}{m} \quad (2)$$

where

s = standard deviation of seven lowest concentration samples
m = slope of the calibration curve

Results and discussion

ESI is a relatively soft ionization technique, producing primarily protonated molecular species. Figure 1 shows the ESI mass spectrum for ketamine including the expected isotope pattern for a molecule with a single chlorine atom. While this is useful for determining the molecular weight of the analyte, it is not sufficient, even with the HPLC retention time, to specifically identify an analyte of forensic interest.[22] We therefore employed multiple reaction monitoring (MRM) to increase the selectivity of the method. By continually isolating the ketamine protonated molecular species at *m/z* 237.7 (alternating with the internal standard at *m/z* 241.7) and observing the fragmentation product ions, the known fragmentation pattern for ketamine (Figure 2) could be monitored. This product spectrum, along with the protonated molecular ion *m/z* and the HPLC retention time allowed for unambiguous identification of ketamine in the beverage residues.

Once all data collection was complete, the averaged ketamine peak values for each concentration (y) were plotted against the concentrations (x) to create a linear calibration curve for each sample type (Figure 3). The R^2 values for the resulting linear regression are presented in Table 1. The computed limit-of-detection (LOD) and limit-of-quantitation (LOQ) values for each sample type are also presented in Table 1. Common oral ketamine doses range between 75 and 300 mg.[23] A 12-oz beverage spiked with 100 mg of ketamine would result in a drug concentration of 1.19 nmol/μl (0.28 mg/ml). As can be seen in Table 1, using LC-MS/MS with selected reaction monitoring of the *m/z* 219.9 fragment from the *m/z* 237.7 precursor ion for quantitation, ketamine could be detected at concentrations 200–2000 times lower than the concentration normally used in a date-rape situation, depending on the beverage and sample type. Ketamine quantitation could be done at concentrations 60–600 times lower than used in a date-rape. Although not all possible beverages have been evaluated, beer is

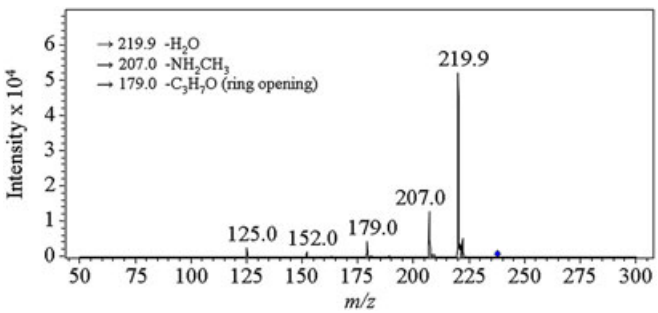

Figure 2. Product ion spectrum resulting from fragmentation of the ketamine protonated molecular species at m/z 237.7.

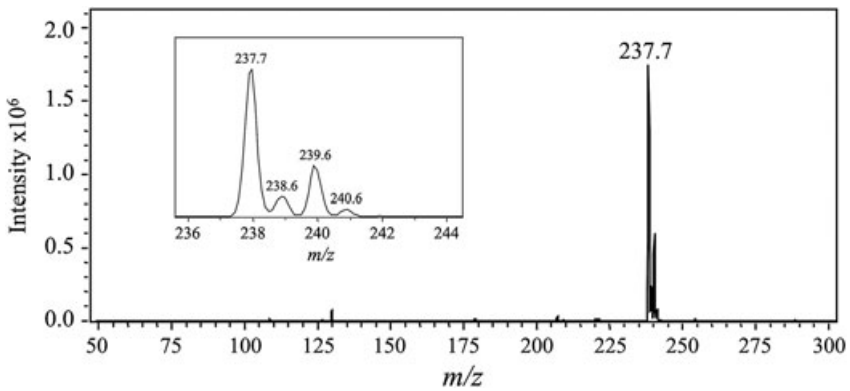

Figure 1. Electrospray ionization mass spectrum of ketamine. Inset is expanded region corresponding to protonated molecule, showing expected isotope pattern. Predicted isotope ratios (left to right):100%, 15%, 33%, 5%, observed ratios: 100%, 14%, 34%, 5%.

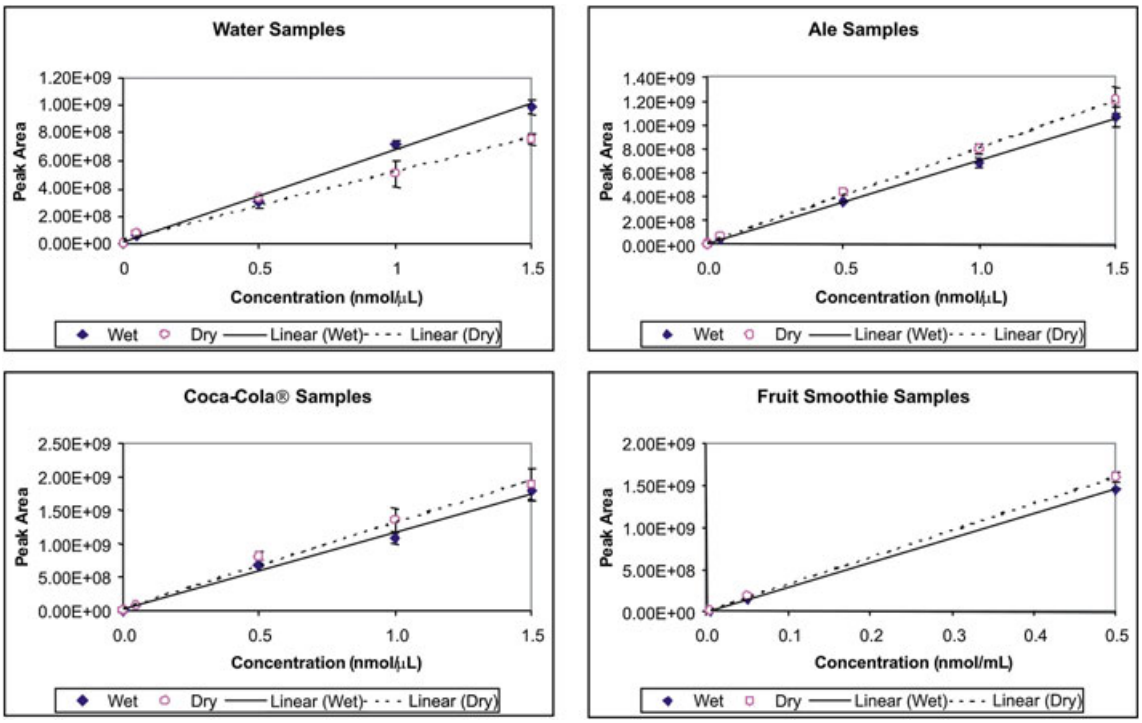

Figure 3. Calibration curves for the detection of ketamine in various beverage residues. Note, six calibration points were used for all studies except for the fruit smoothie, where the linear relationship ended at concentrations above 0.5 nmol/μL.

Table 1. Statistical figures of merit for various ketamine samples

Beverage	Sample Type	Calibration curve R^2 value	LOD nmol/μl (mg/ml)	LOQ nmol/μl (mg/ml)
Water	Wet	0.9949	5.76×10^{-4} (1.36×10^{-4})	1.92×10^{-3} (4.54×10^{-4})
	Dry	0.9881	6.09×10^{-4} (1.44×10^{-4})	2.03×10^{-3} (4.81×10^{-4})
Ale	Wet	0.9992	5.19×10^{-3} (1.23×10^{-3})	1.73×10^{-2} (4.09×10^{-3})
	Dry	0.9989	3.37×10^{-3} (7.98×10^{-4})	1.12×10^{-2} (2.65×10^{-3})
Coca Cola®	Wet	0.9925	2.06×10^{-3} (4.88×10^{-4})	6.86×10^{-3} (1.62×10^{-3})
	Dry	0.9914	1.66×10^{-3} (3.93×10^{-4})	5.53×10^{-3} (1.31×10^{-3})
Fruit Smoothie	Wet	0.9997	1.94×10^{-3} (4.59×10^{-4})	6.48×10^{-3} (1.53×10^{-3})
	Dry	0.9999	3.08×10^{-3} (7.29×10^{-4})	1.02×10^{-2} (2.41×10^{-3})

among the more chemically complex beverages and therefore significant problems are not anticipated with other beverages.

Conclusions

We have demonstrated that direct analysis using LC-MS of beverage residues is simple, fast, selective, and sensitive enough to determine if the beverage has been spiked with ketamine at concentrations less than normally used in a date-rape scenario. The method described does not require any prior sample preparation, although the solid particulate matter must be removed from the blended fruit smoothie beverage. This method could likely be modified to allow the detection of even lower concentrations of ketamine if significant sample preparation steps were added, such as solid-phase extraction (SPE) or sample preconcentration. It should be noted that while 5 ml of water was used to collect the ketamine from the beverage residues, only 5 μl of this solution was used in each analysis. A simple solvent evaporation step would likely allow us to observe smaller concentrations. These preconcentration or SPE steps were not incorporated into the method as we wanted to make the method as rapid and simple as possible. In addition, as the described method was able to identify the presence of ketamine at concentrations approximately 200–2000 times lower than those normally used in date-rape scenarios, there is not a compelling reason to make the method more sensitive by adding additional sample preparation steps. The method described here should be applicable, with possible slight modifications, to other date-rape drugs such as rohypnol, GHB, and ecstasy. As there is minimal sample preparation, and no solvent extraction or derivatization steps, using LC-MS/MS may be an attractive alternative to GC-MS for the analysis of ketamine in beverages and beverage residues.

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