Extraction and analysis of ketamine (a rave drug) from spiked fruit juices using MEPS-MS for forensic purposes

Sandeep Singh Sahota[1], Jaswinder Singh[1], Gulsan Bansal[2] and Rakesh Kumar Garg[1,*]

[1]Department of Forensic Science, and
[2]Department of Pharmaceutical Sciences and Drugs Research, Punjabi University, Patiala 147 002, India

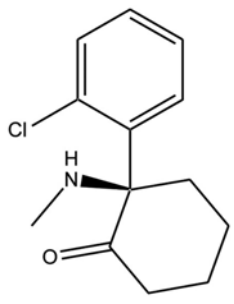
Micro extraction packed sorbent (MEPS) containing C8 and SCX (strong cation exchange) is employed to extract ketamine, a rave drug from fruit juices. The eluant was detected by mass spectral analysis wherein the drug was detected as a parent as well as some prominent fragment ions. The resultant MEPS-MS method proved very efficient and rapid. The mass spectrum of the eluant showed the parent ion peak (MH^+) of ketamine at *m*/*z* 238 along with (MH^{+2+}) peak (30% of MH^+) due to chlorine atom in the drug molecule. The daughter ions at *m*/*z* 220 and 207 were possible to be formed from the MH^+ by loss of water and methylamine molecule respectively. Another daughter ion at *m*/*z* 179 was proposed to be formed by the loss of CO (28 mass units) from *m*/*z* 207 whereas *m*/*z* 125 was assigned to a chlorobenzyl cation. Based on the mode of fragmentation of drug to *m*/*z* 220 and 207 and their abundances, these ions can be used for quantitative analysis through selective reaction monitoring (SRM).

Keywords: Date rape, extraction, juices, ketamine, mass spectrometry, MEPS.

KETAMINE, a member of cyclohexanone derivatives, is a common intravenous anaesthetic used in anaesthesia induction and short-time surgeries (Figure 1). It is a type of recreational drug, known as 'Special-K' and 'Kit-Kat'[1,2], which leads to a lot of drug-related crimes. It is misused by teens and young adults at dance clubs and events known as raves. According to the World Drug Report 2011[3], seizures of ketamine tripled over the years 2005–2009 and in volume terms, some 20 times larger than ecstasy seizures in Asia in 2009. It has become popular as well among young users and is often used as a cocktail with ecstasy[4].

Beverages are the most common medium for misuse of ketamine because of the latter's odourless and tasteless properties. It could be easily mixed into beverages and fruit juices without being detected by the victim. These properties make it an effective 'date rape drug'. In humans, a single dose of the drug (approximately 6–13 mg kg^{-1}) induces amnesia which may be evident for 1–2 h when the victim is not able to speak or move[3]. Even the victims may not be able to recall events that took place while they are under the influence, making it an even more effective date rape drug. The forensic expert needs to examine these beverages to know the source of the drug and its feasibility of detection under which the crime was committed, to link it with the crime scene.

Various studies have reported on the extraction of ketamine from urine[2,5–7], oral fluid[7], hair[1,8,9], canine plasma[10] and blood[11]. But no significant study was done on extraction of ketamine from the beverages and fruit juices. Acikkol *et al.*[12] have determined benzodiazepines and ketamine from alcoholic and non-alcoholic beverages by GC-MS using chloroform: isopropanol (1 : 1, v/v) for extracting the drug from drinks. Dubey *et al.*[11] used traditional sodium sulphate solvent extraction technique for extracting ketamine from blood sample. This technique requires solvent and buffers and is time consuming. Apart from the solvent–solvent extraction technique, various other sample preparation techniques are available in literature for drug extraction from the complex matrix such as liquid–liquid extraction method (LLE), solid phase extraction (SPE) and solid phase micro extraction (SPME). The LLE method requires solvent or solvent mixtures of high polarity and these often yield emulsions. For analytes of more polarity, recovery and sample purity is a further difficult task. SPE is the most suitable technique for extraction of drug because of its ability to efficiently retain highly functionalized compounds from aqueous samples and then to release those into organic solvents on elution. SPE methods are useful for complex biological samples because the main requirements of extraction (matrix exchange, desalting, removal of macromolecules and highly polar compounds) are well matched to the properties of the sorbent[13]. Micro Extraction Packed sorbent (MEPS) is a modern technique for extracting drugs from varied matrices. Invented and developed at AstraZeneca, Sodertalje, Sweden, MEPS offers many advantages over SPE and LLE such as simplicity, speed, non-solvent consumption and sensitivity. It is a miniaturized SPE and a logical extension of SPE for handling biological fluids because of small operating volumes which



(RS)-2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone

Figure 1. Structure of ketamine.

*For correspondence. (e-mail: rkgvpbi11@yahoo.com)

reduces the size of sample required. Lafay *et al.*[14] have compared MEPS and SPE/LLE for determining cotinine in urine samples for regulatory and passive smokers. The total time of SPE/LLE extraction was 24 h while MEPS took only 5 min. Moreover, the single MEPS sorbent could be used for more than 200 extractions without any loss of extraction capacity; the solvents in SPE and LLE are not reusable. Further, linearity, precision, limit of detection (LOD) and limit of quantification (LOQ) are the same for both methods.

In MEPS, the sorbent (1–4 mg) is either inserted into the syringe (100–250 µl) barrel as a plug or between the needle and the barrel as a cartridge. The cartridge bed can be packed or coated to provide selective and suitable sampling conditions. The range of sorbent material includes silica based (C2, C8, C18), strong cation exchange (SCX) using sulfonic acid bonded silica, restricted (RAM), hilic, carbon, polystyrene-divinylbenzene copolymer (PS-DVB) or molecular imprinted polymers (MIPs)[15,16].

Mass spectrometry (MS) is an analytical technique that helps to identify the amount and type of chemicals present in a sample by measuring the mass-to-charge ratio and abundance of gas-phase ions (Figure 2). It is considered as the best technique to identify any component present in the sample. It has three stages – ionization source, mass analyser and detector. All these operate under vacuum. At the ionization source, the sample (liquid or gas) injected changes to vapour state and is ionized by the bombardment of ions resulting in fragmentation of the sample molecule. At the second stage, ions generated from the sample are separated according to their mass-to-charge ratio under high magnetic field. At the last stage, detector captures the signal as the ions pass through the mass detector. The final result is obtained on the computer screen in the form of graph having two axes – *X* axis (mass to charge ratio) and *Y* axis (relative abundance of mass fragments). This technique is the most versatile technique available today and together with other separating techniques it helps in absolute identification of the compound.

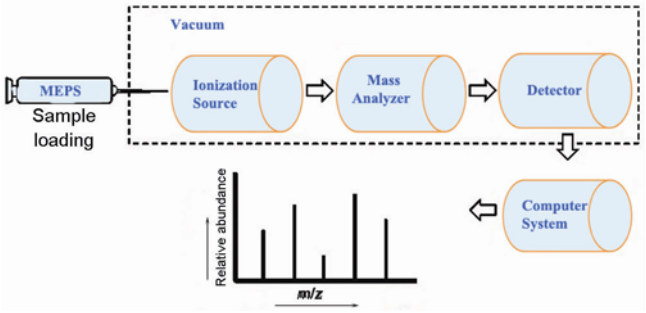


Figure 2. Diagrammatical representation of mass spectrometry.

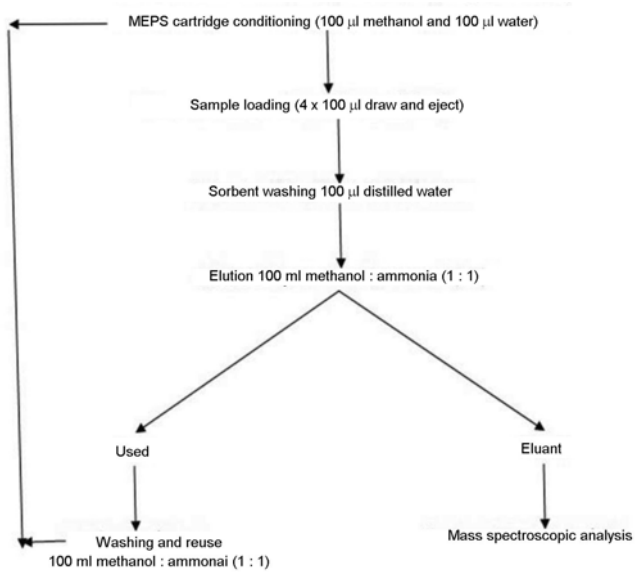


Figure 3. Extraction process of ketamine using MEPS.

The present study was designed to detect ketamine through mass spectrometry after its extraction from fruit juices with MEPS. Apart from extraction, the recovery strength is also studied by analysing the different concentrations of the eluant after passing through the extraction column. This combination of MEPS-MS techniques offered a very sensitive and efficient method for ketamine analysis.

Ketamine hydrochloride injection (Ketmin®, Themis Medicare Limited, Haridwar, India (Batch no. – KME242, Mfd. – 07-2012, Expiry – 06-2014 with label claim of ketamine 50 mg ml^{-1}) was bought from a local chemist shop. Three fruits juices (Real mixed fruit juice Batch no. – NB3008D, Mfd. by Dabur Nepal, Bara, Nepal; Real Guava juice Batch no. GD300D-3 and Minute Maid® Nimbu fresh juice, Mfd. by Kandhari Beverages, Fatehgarh Sahib, Punjab, India Batch no. BH103) were purchased from a local grocery shop and used as samples for extraction. Methanol and ammonia were purchased from Loba Chemie (Mumbai, India). MEPS with C-8 + SCX phase sorbent were purchased from SGE analytical (Australia). Mass spectral analysis was performed using

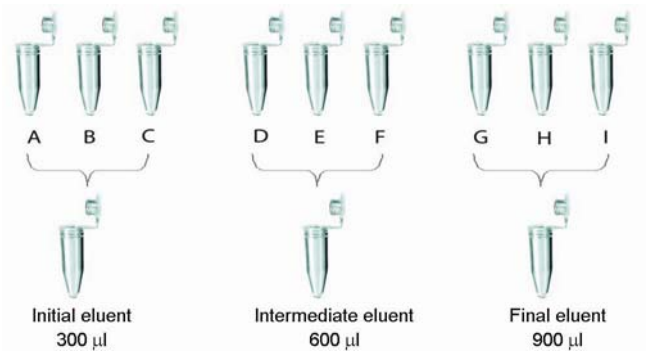


Figure 4. Experimental set-up for determining strength of the recovery of ketamine from eluant.

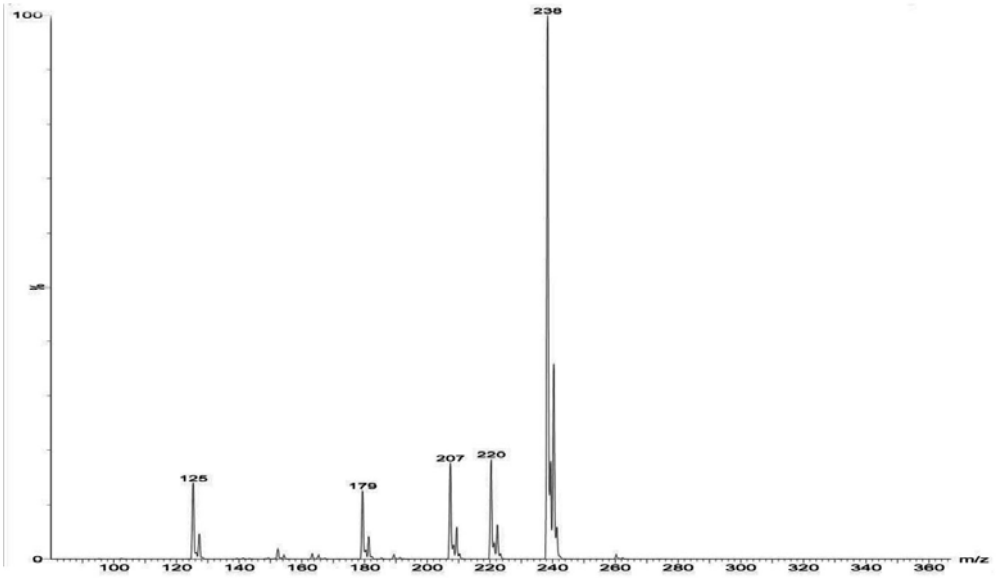


Figure 5. Mass spectrum of ketamine after extraction from spiked juices.

Q-TOF mass spectrometer (QSTAR XL, Applied Biosystems/MDS Sciex, Foster City, CA, USA), equipped with an ESI source. The data acquisition was done by Analyst QS software (Applied Biosystems).

Ketamine hydrochloride (0.5 ml) was spiked in 50 ml of each fruit juice using syringe. After spiking, the sample was filtered using Whatman filter paper to reduce the viscosity of the sample. Ketamine was extracted from spiked juices with MEPS containing C-8 + SCX phase sorbent (Figure 3). MEPS cartridge was conditioned with 100 µl of methanol followed by 100 µl of water. A 100 µl of the sample was flushed through the conditioned MEPS with a syringe. The flushing was repeated four times. The sample loaded MEPS was washed with 100 µl of water to elute unwanted components of the matrix while keeping the drug immobilized on the sorbent. The clean loaded MEPS was run with 100 µl of methanol: ammonia (1 : 1) to elute the drug from the sorbent. The eluant was subjected to mass spectrometry analysis and the used MEPS was washed with 100 µl of water followed by 100 µl of ammonia: methanol (1 : 1). The washing was repeated four times and the washed MEPS could be reused up to 200 times for extraction.

In the present study, an experiment was also carried out to determine the concentration of ketamine in every 300 µl eluant. An experiment was performed until no result was obtained for detecting the compound in the eluant. The initial, intermediate and final eluants were subjected to mass spectroscopic analysis (Figure 4).

The eluant was analysed for detecting ketamine through mass spectrometry analysis using a positive mode of electrospray ionization (+ESI). The operating conditions were optimized as follows: MSD was operated in the (ES+) mode with 60 eV and in full scan mode (*m*/*z* 50–600). The typical source conditions were: capillary temperature – 160°C, capillary voltage – 15–20 V, source voltage – 5 kV, shealth gas flow – 1.35 l min^{-1} and auxillary gas flow – 6 l min^{-1}. The mass spectrum was recorded in full scan mode (*m*/*z* 50–600).

Ketamine is a basic drug and it is supplied as hydrochloride salt. Due to its moderately polar nature, it exists as an ammonium salt and a sorbent combination of C8 and SCX was employed for its elution. pK_a value of SCX is <1 and is negatively charged. In an acidic solution (juice pH 3.5), ketamine possesses a positive charge and is thus retained by ionic interactions with the SCX bonded phase. This strong ionic retention mechanism allowed the sorbent to be washed with the polar solvent, water, which effectively removes anionic and neutral interferences without affecting the recovery of ketamine. A mixture of organic solvent (methanol) and ammonia in a ratio 1 : 1 disrupts the analyte-sorbent interaction resulting in the elution of the drug. Ketamine was successfully extracted from spiked juice samples. Multiple flushing of the MEPS with sample helps in increasing the extraction efficiency and sensitivity of the method. To determine the strength of recovery of ketamine with every flushing of the analyte through the recovery solvent, a method was developed (Figure 4). It was observed that 75% of the analyte was eluted in the first 300 µl of the elutant whereas the remaining 25% was eluted in the next 300 µl and no result was obtained in the further analysis of the elutant.

Mass spectrometry is an excellent analysis tool that detects the molecular concentration as low as one picogram. The mass spectrum of the eluant showed the parent ion peak (MH^+) of ketamine at *m*/*z* 238 along with (MH^{+2+}) peak (30% of MH^+) due to chlorine atom in the

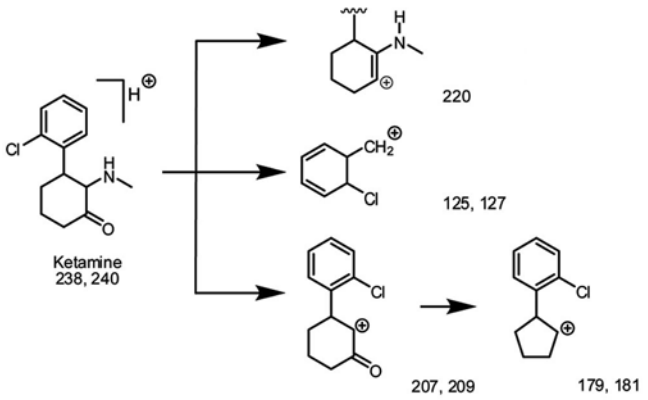


Figure 6. Mass fragmentation of ketamine in the eluant.

drug molecule (Figure 5). The daughter ions (fragment peak produced from parent ion peak) at *m*/*z* 220 and 207 were possible to be formed from the MH^+ by loss of water and methylamine molecule respectively. Another daughter ion at *m*/*z* 179 was proposed to be formed by loss of CO (28 mass units) from *m*/*z* 207 whereas *m*/*z* 125 was assigned to a chlorobenzyl cation (Figure 6). All the four fragment ions were accompanied with a corresponding 2 mass units higher isotopes peaks due to the chlorine atom retained in the fragments. Based on the mode of fragmentation of drug to *m*/*z* 220 and 207 and their abundances, these ions can be used for quantitative analysis through selective reaction monitoring (SRM).

The present study reveals the application of modern extraction technique, MEPS, to extract ketamine from fruit juices followed by detection with mass spectrometry. It was concluded that the complete extraction was performed in 600 µl of elutant, 75% of the analyte was eluted in the first 300 µl of the elutant whereas the remaining 25% was eluted in the next 300 µl and no result was obtained in the further analysis of the elutant. This hyphenated technique, MEPS-MS has been found to be an efficient and rapid method for qualitative analysis of ketamine from varied types of juices and this study is quite useful for forensic investigations. The method can be further exploited for its quantitative applications.

1. Tabernero, J., Felli, M. L., Bermejo, A. M. and Chiarotti, M., Determination of ketamine and amphetamines in hair by LC/MS/MS. *Anal. Bioanal. Chem.*, 2009, **395**, 2547–2557.
2. Moore, K., Sklerov, J., Levine, B. and Jacob, A. J., Urine concentrations of ketamine and norketamine following illegal consumption. *J. Anal. Toxicol.*, 2001, **25**, 583–588.
3. NIDA Community Drug Alert Bulletin Club Drugs; 2004, http://archives.drugabuse.gov/ClubAlert/clubdrugalert.html last cited on 27.10.2015.
4. Kulsudjarit, K., Drug problem in southeast and southwest Asia. *Ann. N. Y. Acad. Sci.*, 2004, **1025**, 446–457.
5. Xiong, J., Chen, J., He, M. and Hu, B., Simultaneous quantification of amphetamines, caffeine and ketamine in urine by hollow fiber liquid phase microextraction combined with gas chromatography-flame ionization detector. *Talanta*, 2010, **82**, 969–975.
6. Chou, S. L., Yang, M. H., Ling, Y. C. and Giang, Y. S., Gas chromatography-isotope dilution mass spectrometry preceded by liquid–liquid extraction and chemical derivatization for the determination of ketamine and norketamine in urine. *J. Chromatogr. B Biomed. Sci. Appl.*, 2004, **799**, 39–50.
7. Cheng, W. C., Ng, K. M., Chan, K. K., Mok, V. K. K. and Cheung, B. K. L., Roadside detection of impairment under the influence of ketamine – evaluation of ketamine impairment symptoms with reference to its concentration in oral fluid and urine. *Forensic Sci. Int.*, 2007, **170**, 51–58.
8. Parkin, M. C., Longmoore, A. M., Turfus, S. C. and Braithwaite, R. A., Detection of ketamine and its metabolites in human hair using an integrated nanoflow liquid chromatography column and electrospray emitter fritted with a single porous 10 µm bead. *J. Chromatogr. A*, 2013, **1277**, 1–6.
9. Favretto, D., Vogliardi, S., Stocchero, G., Nalesso, A., Tucci, M., Terranova, C. and Ferrara, S. D., Determination of ketamine and norketamine in hair by micropulverized extraction and liquid chromatography – high resolution mass spectrometry. *Forensic Sci. Int.*, **2013**, 226, 88–93.
10. Niedorf, F., Bohr, H. H. and Kietzmann, M., Simultaneous determination of ketamine and xylazine in canine plasma by liquid chromatography with ultraviolet absorbance detection. *J. Chromatogr. B. Biomed. Sci. Appl.*, 2013, **791**, 421–426.
11. Dubey, P., Shukla, S. K. and Gupta, K. C., Stability of ketamine in human biological samples: effect of temperature. *Int. J. Med. Toxicol. Legal Med.*, 2013, **15**, 33–36.
12. Acikkol, M., Mercan, S. and Karadayi, S., Simultaneous determination of benzodiazepines and ketamine from alcoholic and nonalcoholic beverages by GC-MS in drug facilitated crimes. *Chromatographia*, 2009, **70**, 1295-1298.
13. Rehim, M. A., Microextraction by packed sorbent (MEPS): a tutorial. *Anal. Chim. Acta*, 2011, **701**, 119–128.
14. Lafay, F., Vulliet, E. and Waton, F. M. M., Contribution of microextraction in packed sorbent for the analysis of cotinine in human urine by GC-MS. *Anal. Bioanal. Chem.*, 2010, **396**, 937–941.
15. Noche, G. G., Laespada, M. E., Pavon, J. L., Cordero, B. M. and Lorenzo, S. M., Microextraction by packed sorbent for the analysis for pharmaceutical residues in environmental water samples by *in situ* derivation programmed temperature vaporizer-gas chromatography mass spectrometry. *J. Chromatogr. A*, 2011, **1218**, 9390–9396.
16. Altun, Z., Rehim, M. A. and Blomberg, L. G., New trends in sample preparation: on-line microextraction in packed syringe (MEPS) for LC and GC applications Part III: Determination and validation of local anesthetics in human plasma samples using a cation-exchange sorbent and MPES-LC-MS-MS. *J. Chromatogr. B. Sci Appl.*, 2004, **813**, 29–135.

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