Forensic Science International 248 (2015) 119–123

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Cut-off proposal for the detection of ketamine in hair

A. Salomone [a,*], E. Gerace [a], P. Diana [a], M. Romeo [c], V. Malvaso [c], D. Di Corcia [a], M. Vincenti [a,b]

[a] Centro Regionale Antidoping "A. Bertinaria", Regione Gonzole 10, 10043 Orbassano, Turin, Italy
[b] Dipartimento di Chimica, Università degli Studi di Torino, via P. Giuria 7, 10125 Turin, Italy
[c] Centro Grandi Ustionati-Ospedale CTO, A.O.U. Città della Salute e della Scienza di Torino, via G. Zuretti 29, 10126 Turin, Italy

ARTICLE INFO

Article history:
Received 28 May 2014
Received in revised form 16 December 2014
Accepted 31 December 2014
Available online 8 January 2015

Keywords:
Hair
Cut-off
Ketamine

ABSTRACT

Ketamine is a powerful anesthetic drug used in both human and veterinary surgery, but it is also commonly misused because of its psychotropic properties. Since the abuse of this drug has been reported in many countries worldwide, its determination in hair samples is offered as a specialist test by hundreds of laboratories. However, unlike other common drugs of abuse, a cut-off level for ketamine in hair has not been fixed yet. Therefore, aim of this study is to propose a concentration value for ketamine in hair analysis, in order to discriminate between chronic and occasional use, and between active use and external contamination. After considering the chemical properties of this molecule, and the experimental data collected in our laboratory or reported in several other published studies, we propose a cut-off level of 0.5 ng/mg, as indicative of repeated exposure to ketamine. Additionally, we suggest that the detection of the metabolite norketamine should be mandatory to prove active intake and exclude false positive result from external contamination. Thus, a reasonable cut-off value for norketamine could be fixed at 0.1 ng/mg, while the minimal concentration ratio norketamine/ketamine may be positively established at 0.05.

1. Introduction

Ketamine is a powerful anesthetic drug used in both human and veterinary surgery since the early 1960s. In pharmaceutical preparations, ketamine is normally found as injectable solution, but it can also be obtained as a powder or tablet. It is administered orally or injected [1], but it can also be snorted, or smoked [2,3]. The psychotropic properties of ketamine have induced its parallel misuse as a recreational drug in a variety of social settings, as well as in drug-facilitated sexual assault, owing to its dissociative and sedative properties [4]. The majority of ketamine abusers are likely teens and young working adults [5]. Since the misuse of this drug has been reported in many countries worldwide, its determination in hair samples is offered as a specialist test by hundreds of laboratories, using different analytical techniques [2–13]. Hair analysis currently represents a reliable and well-established means of clinical and forensic investigation, insomuch as it is regularly requested to evaluate drug exposure, to portray drug abuse history and/or withdrawal control, to perform workplace drug testing, driving re-licensing and post-mortem investigations, to ascertain drug-facilitated crimes, and occasional or prenatal exposure to drugs [14,15]. Consequently, the international community (in particular, The Society of Hair Testing) periodically meets to exchange scientific experiences, and establish new protocols and consensus documents in order to provide thorough interpretation of the analytical results and consequent clinical or legal judgments. As a matter of fact, few guidelines about hair testing do exist [16,17], and are constantly revised. In several circumstances, such as in workplace drug testing, accurate correlation between the degree of positive detection in hair specimens and the correct category of ketamine exposure is of extreme importance and may have serious consequences for the investigated subjects. Nevertheless, a specific cut-off value for ketamine in hair has never been proposed, nor it has been suggested for its main metabolite, norketamine, ultimately implying that their mere detection in hair represents a prove of drug exposure. Indeed, norketamine, which is generated by *N*-demethylation of ketamine, reaches blood concentrations similar to ketamine itself [18]. However, the concentration of ketamine in hair is expected to be higher than that of its metabolite, because norketamine is more polar than the parent

* Corresponding author. Tel.: +39 011 90224232; fax: +39 011 90224242; mobile: +39 3489330145.
E-mail address: alberto.salomone@antidoping.piemonte.it (A. Salomone).

http://dx.doi.org/10.1016/j.forsciint.2014.12.030
0379-0738/

drug, and the extent of hair deposition is widely known to correlate with the lipophilicity of the drug.

In the present study, we evaluated a miscellaneous of case reports, analytical results, and published data, either obtained in our laboratory and from peer-reviewed literature, all describing the detection of ketamine and its metabolite norketamine in the keratin matrix. Our aim was to identify a tentative discriminating concentration to be used as a cut-off in hair analysis, in order to distinguish chronic and illicit abuse from occasional use (e.g. for medical reasons) of ketamine, or between active use and external contamination.

2. Materials and methods

2.1. Sample preparation

Only the proximal 0–6 cm segment was analyzed whenever longer head hair samples were collected. Shorter head hair samples, as well as body hair samples, were analyzed in their full length. About 50 mg of hair was twice-washed with dichloromethane (2 mL, vortex mixed for 3 min). The solvent washes were completely removed and discarded, then the hair was dried at room temperature by a gentle nitrogen flow and subsequently cut with scissors into 1–2 mm segments. Hair samples were fortified with 2 μL of internal standard solution yielding a final concentration of 0.4 ng/mg. After the addition of 2 mL of methanol, the samples were incubated at 55 °C for 15 h without stirring. Lastly, the organic phase was collected and an aliquot of 1 μL was directly injected into the UHPLC–MS/MS system.

2.2. Instrumentation

The method normally used in our laboratory to detect the most common drugs of abuse [19] was modified to include ketamine and norketamine. SRM transitions and potential settings for the analytes are presented in Table 1.

The method was fully revalidated according to national and international guidelines [20,21]. Linearity was verified in the interval 0.05–5.0 ng/mg. Whenever the real samples concentrations were found to exceed the highest calibration point, the final extracts were diluted with methanol and re-injected into the system. Limit of Detection (LOD) and Limit of Quantitation (LOQ) for ketamine and norketamine were, respectively, 0.004 and 0.003 ng/mg (LODs) and 0.013 and 0.010 ng/mg (LOQs). Interday precision and accuracy were tested at 0.05 ng/mg, showing that all experimental values were below the acceptable CV and bias limits of ±15%. Matrix effects for ketamine and norketamine were, respectively, +24% and −13%. Laboratory performances are constantly monitored through regular participation to inter-laboratory proficiency tests.

2.3. Detection of ketamine in the published literature

It is well-acknowledged that the concurrent detection of drug metabolites in biological specimens provides evidence of active intake, preventing false positive and misleading interpretation of the analytical results. In the following summary of the inherent published literature, only the articles presenting the detection of ketamine and its metabolite norketamine in real hair samples were included. The analytical techniques most frequently used to detect ketamine and norketamine in hair were GC–MS [2,3,5,13,22,23] and LC–MS/MS (or LC–HRMS) [4,7]. In one case, a specific method using HPLC-Chip–MS/MS [11] was developed. A summary of the reviewed methods is presented in Table 2.

3. Results

All results are summarized in Table 3.

3.1. Case study 1

A 20-years old male, black haired, declared intake of ketamine for recreational purpose. He estimated to have consumed ketamine about 10 times in the 6–8 months before the sampling. His hair length was 4.5 cm. Samples were taken and analyzed in our laboratory, according to the procedure previously described. Calculated concentration of ketamine (K) was 1.87 ng/mg, norketamine (NK) was 0.11 ng/mg, and the NK/K ratio was 0.06.

3.2. Case study 2

In 2013, we found 6 subjects (5 males, 1 female) positive for ketamine (range 0.11–11.4 ng/mg, mean 2.09 ng/mg, median 0.26 ng/mg). Norketamine was detected in all samples (range 0.02–0.71 ng/mg, mean 0.15 ng/mg, median 0.04 ng/mg). The NK/K ratios were in the range 0.06–0.29. Age range was 20–29 years (mean 24.2).

3.3. Case study 3

In 2014, we found 8 subjects (7 males, 1 female) positive for ketamine (range 0.32–7.22 ng/mg, mean 2.75 ng/mg, median 1.96 ng/mg). Norketamine was detected in all samples (range 0.06–0.65 ng/mg, mean 0.31 ng/mg, median 0.25 ng/mg). The NK/K ratios were in the range 0.03–0.53. Age range was 17–32 years (mean 24.1).

3.4. Case study 4

In this study [5], hair samples were obtained from either self-confessed or suspected ketamine abusers. The authors fixed an arbitrary cut-off level at 1 ng/mg. Fifty-one hair samples resulted positive for ketamine, with concentrations varying in the range 0.6–489.0 ng/mg (mean 49.0 ng/mg), whereas the concentration range of norketamine was 0.8–196.3 ng/mg (mean 12.1 ng/mg). The NK/K ratio varied from 0.05 to 0.84 (mean 0.33). According to voluntary confessions from the abusers who snorted the drug about once a week, the resulting hair concentration of ketamine was in the range 1.1–42.7 ng/mg (mean 9.9 ng/mg).

Table 1
SRM transitions and corresponding potentials for the target compounds.

Compound	RT (min)	SRM transitions (m/z)[a]	DP (V)	CE (V)	CXP (V)
Cocaine-d3 (ISTD)	1.5	**307.1 → 185.1**	71	27	11
		238.0 → 125.0		35	11
Ketamine	2.2	238.0 → 207.0	45	19	9
		238.0 → 179.0		23	15
Norketamine	2.6	**224.1 → 125.0**	40	35	11
		224.1 → 207.1		19	9

[a] Target transitions used for quantitation are marked in bold.