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REVIEW ARTICLE

Identification of ketamine and its metabolites in biological samples: A systematic review

SANAZ PASHAPOUR[1,*]

[1]Department of Forensic Toxicology, Legal Medicine Research Center, Legal Medicine Organization, Tehran, Iran

Abstract

Background: While numerous studies have delved into the analysis of ketamine (KET) in biological samples, this forthcoming study undertook a systematic review of diverse methods for identifying KET in various biological samples to attain a more precise estimation.
Methods: Research articles published from 2004 to September 30, 2024, were retrieved from PubMed, Scopus, and Google Scholar databases. A methodical search was conducted using English search terms, such as "Ketamine" OR "K" OR "ketamine hydrochloride" OR "Norketamine" OR "NK" OR "N-desmethyl ketamine" OR "2-amino-2-(2-chlorophenyl)cyclohexan-1-one" OR "(+-)-Hydroxynorketamine" OR "HNK" OR "Dehydronorketamine" OR "DNK" OR "Metabolites" AND "Analysis" OR "Analytical techniques" OR "Analytical innovations" OR "Methods" OR "Identification" OR "Gas chromatography" OR "Liquid chromatography" OR "Mass Spectrometry" AND "Biological samples" OR "Biological matrices". The selection criteria were established according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.
Results: Out of 3,450 articles identified in the initial systematic search, 50 met the inclusion criteria and were investigated in this study. The findings revealed that advanced hyphenated analytical methods combined with mass spectrometry (MS), such as gas chromatography-MS (GC-MS), liquid chromatography-MS (LC-MS)were prominent. Additionally, liquid-liquid extraction and solid-phase extraction were the prevailing methods employed for KET sample preparation.
Conclusion: Overall, a comprehensive understanding of the available identification methods for KET and its metabolites is essential for accurate and reliable analysis in various fields, including clinical research, forensic investigations, and drug monitoring programs.

Keywords: Ketamine, Metabolites, Mass Spectrometry, Chromatography

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INTRODUCTION

Ketamine (KET), also known as 2-(2-chlorophenyl)-2-(methylamino)-cyclohexanone, was created by Calvin Stevens in 1962 at Parke-Davis in Michigan as an alternative to phenylcyclohexyl piperidine. KET is classified as a dissociative anesthetic and functions by blocking nerve signals in the brain [1-6]. It is a medication primarily used to start maintaining anesthesia and thus providing pain relief, sedation, and memory loss. Misuse or overdose of KET can lead to various toxic effects. KET toxicity manifests acutely with hallucinations, confusion, hypertension, tachycardia, respiratory depression, and, at very high doses, coma or death; chronically, it can cause bladder inflammation, cognitive deficits, tolerance with dependence, and lasting mood or psychotic disorders. Accurate detection of KET and its metabolites in biological samples is vital for guiding treatment and forensic evaluations [2, 3]. In Europe, KET was found in illicit mixtures with stimulants such as 3,4-methylenedioxymethamphetamine starting in 1990. Its illicit usage dates back to the late 20th century, and its recreational use has increased since then. In China, KET has gained popularity in the drug market, becoming the third most commonly used illicit substance by 2011. The total amount of KET confiscated globally rose from an average of 3 tons annually between 1998 and 2008 to 10 tons between 2009 and 2014 and further increased to 15 tons per year from 2015 to 2017. KET was included in the *Drug* Enforcement Administration's emerging drugs list in 1995 and was classified as a Schedule II Controlled Substance in the United States in 1999 [4-9]. This medication undergoes hepatic metabolism to produce active metabolites, with a half-life of around 2–3 hours. It is primarily metabolized in the liver by the enzymes CYP3A4 and CYP2B6 to form norketamine (NK), an active metabolite that contributes to KET's effects. NK is further metabolized to hydroxynorketamine. These metabolites are eventually excreted in the urine. The identification of KET and its metabolites in biological samples is a crucial area of research in pharmacology and toxicology [1, 2, 4, 6, 8, 10]. Understanding the pathways of KET metabolism and

*Correspondence to: Dr. Sanaz Pashapour, Department of Forensic Toxicology, Legal Medicine Research Center, Legal Medicine Organization, Tehran, Iran.
Email: pashapour.sanaz@yahoo.com, Tel: +98 9372947927.

accurately identifying these metabolites in biological samples are essential for medical purposes, forensic investigations, and drug abuse monitoring. Various analytical techniques are employed for the identification of KET and its metabolites, such as chromatography, including gas chromatography [GC] and liquid chromatography [LC], coupled with mass spectrometry (MS). These methods allow for the sensitive and specific detection of KET and its metabolites in complex biological matrices such as blood, urine, and tissue samples [2, 3]. Although previous studies have explored the identification of KET and its metabolites in various biological matrices, to the best of our knowledge, a comprehensive review that consolidates and evaluates all existing methods and findings has been lacking. Accordingly, this systematic review aims to fill this gap by providing a detailed analysis of the techniques used for detecting KET and its metabolites, examining their reliability, sensitivity, and application in clinical and forensic settings. The novelty of this work lies in its broad scope, integrating the most recent advancements in analytical methodologies and their relevance in understanding the pharmacokinetics of KET.

METHODS

Search Strategy

This systematic review addressed recent advancements in analytical methods utilized for identifying and measuring KET and metabolites in toxicological examinations. The research articles were sourced from PubMed, Scopus, and Google Scholar databases, spanning from 2004 to September 30, 2024. A methodical search was conducted using different English search terms, including “Ketamine” OR “K” OR “ketamine hydrochloride” OR “Norketamine” OR “NK” OR “N-desmethyl ketamine” OR “2-amino-2-(2-chlorophenyl)cyclohexan-1-one” OR “(+-)-Hydroxynorketamine” OR “HNK” OR “Dehydronorketamine” OR “DNK” OR “Metabolites” AND “Analysis” OR “Analytical techniques” OR “Analytical innovations” OR “Methods” OR “Identification” OR “Gas chromatography” OR “Liquid chromatography” OR “Mass Spectrometry” AND “Biological samples” OR “Biological matrices”. The selection criteria were established according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.

Criteria for Article Selection

Irrelevant articles were excluded after reviewing all abstracts. Potentially relevant articles were identified, followed by obtaining their full texts.

Inclusion criteria

The selection criteria included studies focusing on the identification and analysis of KET in human biological samples and articles available in the English language.

Exclusion criteria

Articles that did not address the identification and analysis of KET in human biological samples, non-English articles, animal studies, in vitro studies, meta-analyses, duplicate articles, and articles that did not align with the specific focus on KET and its metabolites in biological samples.

RESULTS

Data Extraction

During the initial search phase, 3,450 articles were examined, resulting in 1,800 unique articles after removing duplicates. Subsequently, 1,000 articles progressed to the next stage after title screening. Among them, 800 lacked sufficient data on method validation and were excluded from the investigation. Ultimately, 50 articles meeting the inclusion criteria were selected for the study (Figure 1). Table 1 presents the characteristics of analytical methods extracted from these 50 studies performed on ketamine and metabolite analysis in biological samples.

Importance of ketamine analysis in biological samples in clinical and forensic toxicology

KET is used as an anesthetic and analgesic in medical investigations [2]. The analysis of KET in biological samples plays a vital role in both clinical and forensic toxicology by aiding in patient treatment, forensic investigations, pharmacokinetic studies, and research endeavors. Monitoring its levels in biological samples helps in determining the appropriate dosage for patients and assessing their response to treatment. KET abuse can have serious health implications, and analyzing its levels in biological samples helps in detecting misuse or overdose cases. In cases of suspected drug-related deaths, analyzing KET levels in postmortem samples can provide valuable information on the cause of death. KET is sometimes involved in criminal activities, such as drug trafficking. Understanding how KET is metabolized and eliminated from the body is essential for determining its effects and potential toxicity [11-14].

Types of Biological Samples in Ketamine Analysis

Blood sample

KET detection in blood samples involves the analysis of blood to identify the presence of KET or its metabolites. Detecting KET in the blood samples of patients in emergency rooms can help medical professionals provide appropriate treatment. In cases of drug-related crimes or accidents, KET detection in blood samples can provide evidence of drug use or abuse. Some workplaces conduct drug testing, including screening for KET, to ensure a safe working environment and compliance with regulations. Detecting KET levels in the blood samples of research participants is important for monitoring its effects and ensuring safety [8, 10, 15, 16]. Among the studies included in this review, 10 were conducted on blood samples. For example, Magdalena et al. developed and validated the detection of KET in blood samples using capillary electrophoresis coupled with a mass spectrometer. In this study, the assay was linear in the range of 25–300 ng/mL. The limit of detection (LOD = 6.0 ng/mL) and the limit of quantification (LOQ = 19.8 ng/mL) were determined [17]. In another study, Sara Odoardi et al. identified many drugs and metabolites, such as opiates, methadone, fentanyl and analogues, cocaine, amphetamines, lysergic *acid* diethylamide, KET, and NK in blood samples using ultra-high performance LC (UHPLC)–MS/MS. In this study, the LOD and LOQ for KET and NK were reported to be 0.5 ng/mL and 2 ng/mL, respectively [18].

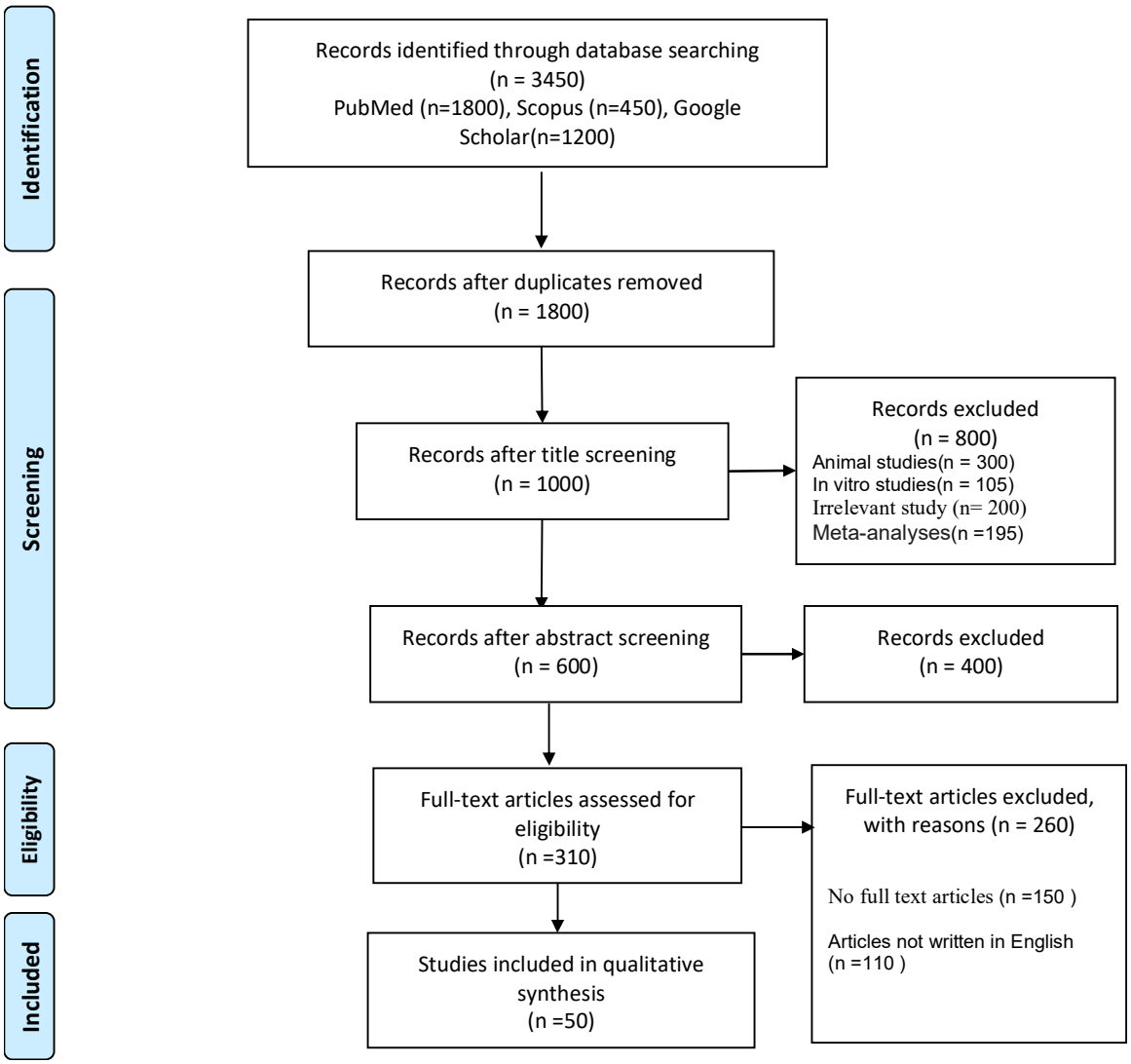

Figure 1. The process of selecting eligible articles

Table 1. A summary of selected studies on ketamine and metabolite analysis in biological samples

First Author (Publication Year)	Biological Sample(s)	Sample Preparation Method	Instrumental Method	Analyte Type and Recovery (%)	LOD	LOQ	Reference No.
Sara Odoardi et al. (2014)	Blood	DBS/MAE	UHPLC–MS/MS	KET (86) NK (81)	0.5 ng/mL 0.5 ng/mL	2 ng/mL 2 ng/mL	[18]
HUEI-RU LIN et al. (2005)	Urine	LLE	GC-MS	KET NK DHNK	1 ng/mL 5 ng/mL 20 ng/mL	5 ng/mL 10 ng/mL 40 ng/mL	[21]
Ankit Rochani et al. (2020)	Plasma	LLE	LC–MS	KET NK DNK	10 ng/mL 300 ng/mL 410 ng/mL	20 ng/mL 320 ng/mL 470 ng/mL	[35]
Kaoqi Lian et al. (2012)	Urine	LLE	GC-MS	KET (98.4)	0.01 µg /mL	0.04 µg/ mL	[2]
Ping Xiang et al. (2006)	Hair	LLE	GC–MS	KET NK	0.02 ng/mL 0.02 ng/mL	0.05 ng/mL 0.05 ng/mL	[3]
Yanshuxian Liu et al. (2020)	Hair	SPE	FE-GC–MS	KET (102)	0.7 ng/mL	2 ng/mL	[10]
Samir M. Ahmad et al. (2020)	Urine	BAµE	GC–MS	KET (105) NK (103)	1 µg /L 1 µg /L	5 µg /L 1 µg /L	[4]

Table 1. Continued.

First Author (Publication Year)	Biological Sample(s)	Sample Preparation Method	Instrumental Method	Analyte Type and Recovery (%)	LOD	LOQ	Reference No.
Gilbert Mercieca et al. (2018)	Blood	DLLME	GC–MS	KET (99.6) NK (79.4)	10 ng/mL 10 ng/mL	10 ng/mL 50 ng/mL	[23]
Gilbert Mercieca et al. (2018)	Urine	DLLME	GC–MS	KET (101.8) NK (102)	5 ng/mL 10 ng/mL	10 ng/mL 50 ng/mL	[23]
Beril Anilanmert et al. (2016)	Urine	LLE	LC–MS/MS	KET (93.99) NK (87.52)	7.15 ng/mL 17.70 ng/mL	18.80 ng/mL 38.70 ng/mL	[36]
Su-Lien Chou et al. (2004)	Urine	LLE	GC–MS	KET NK	10 ng/mL 5 ng/mL	15 ng/mL 10 ng/mL	[8]
Jongsook Rhee et al. (2021)	Hair	DLLME	LC–MS/MS	KET NK	0.001 ng/mL 0.001 ng/mL	0.004 ng/mL 0.004 ng/mL	[5]
Liang Menga et al. (2021)	Hair	SPME	LC-MS	KET (94.1) NK (94.4)	0.067 ng/mL 0.067 ng/mL	Not reported	[37]
Paweł Stelmaszczykbet al. (2021)	Blood	DBS/MAE	LC-MS	KET (112.4)	21.1 ng/mL	70.4 ng/mL	[38]
Cindy Ramiole et al. (2017)	Plasma	LLE	LC–MS/MS	KET (99.4)	0.015 ng/mL	0.031 ng/mL	[39]
Pui-Kin So et al. (2013)	Urine Oral fluid	Not reported	WT-ESI-MS	KET (94.1) NK (94.4)	20 ng/mL	50 ng/mL	[40]
André Valle de Bairros et al. (2014)	Urine	HF-LPME	GC–MS	KET (101) NK (94.3) DNK (69.7)	0.25 ng/mL 0.10 ng/mL 0.10 ng/mL	0.50 ng/mL 0.50 ng/mL 0.50 ng/mL	[41]
Adriana S et al. (2016)	Hair	SPE	LC–MS/MS	KET NK	0.008 ng/mL 0.015 ng/mL	0.02 ng/mL 0.04 ng/mL	[42]
Robert Hofstetter et al. (2018)	Urine	LLE	SFE–SFC–MS	KET NK DNK	Not reported	0.5 ng/mL 0.5 ng/mL 0.5 ng/mL	[43]
Norlida Harun et al. (2010)	Hair	MISPE	LC–MS/MS	KET (86.1) NK (88.4)	0.1 ng/mL 0.4 ng/mL	0.18 ng/mL 0.23 ng/mL	[6]
J.M. Matey et al. (2021)	Hair	DLLME	UHPLC-HRMS/MS	KET NK	2 pg/mL 2 pg/mL	20 pg/mL 20 pg/mL	[44]
Jie Cao et al. (2022)	Urine Blood	Not reported	CFIA GC-MS/MS	KET (96.03) KET (116.20)	0.0001 ng/mL 1.4 ng/mL	Not reported	[45]
Lisa Kurzweil et al. (2020)	Blood	SPE	UHPLC–MS	KET (97.7) NK (97) cis-6- HNK (102)	0.08 ng/mL 0.5 ng/mL 0.1 ng/mL	0.5 ng/mL 0.6 ng/mL 0.8 ng/mL	[46]
Georg M et al. (2017)	Urine	DLLME	SFC/UHPLC	KET (91) NK (78) HNK (62) DNK (76)	1 ng/mL 1 ng/mL 3 ng/mL 1 ng/mL	5 ng/mL 5 ng/mL 5 ng/mL 5 ng/mL	[47]
Kaung-Chaun Wang et al. (2005)	Urine	SPE	LC–-MS/MS	KET (90) NK (90)	Not reported	1 ng/mL 1 ng/mL	[24]
T. Legrand et al. (2008)	Plasma	SPE	LC-MS	KET (98.1) NK (99.5)	1 ng/mL 1 ng/mL	4 ng/mL 4 ng/mL	[48]
Ruin Moaddel et al. (2010)	Plasma Urine	SPE	LC–MS/MS	KET NK HNK DNK	Not reported	Not reported	[49]
Huei-Ru Lin et al. (2014)	Urine	Filter 0.22 µm	LC–MS/MS	KET (98.7) NK (119.4) DNK (99.7)	25 ng/mL 10 ng/mL 10 ng/mL	1-37.5 ng/mL	[29]
Mahmoud Hasan et al. (2017)	Urine	LLE	LC–MS/MS	KET (108) NK (107) DNK (97.2) HNK (89.1)	Not reported	0.1 ng/mL 0.1 ng/mL 0.1 ng/mL 0.1 ng/mL	[19]
Maria Esther Rodriguez Rosas et al. (2003)	Plasma	SPE	LC-MS	KET (98)	Not Reported	1 ng/mL	[28]
Ya-Hsueh Wu et al. (2008)	Hair	LLE	GC–MS	KET (72.7) NK (71.7)	0.05 ng/mL 0.05 ng/mL	0.08 ng/mL 0.08 ng/mL	[50]

Table 1. Continued.

First Author (Publication Year)	Biological Sample(s)	Sample Preparation Method	Instrumental Method	Analyte Type and Recovery (%)	LOD	LOQ	Reference No.
Min-Kun Huang et al. (2008)	Urine	SPE	GC–MS	KET (93) NK (93)	0.5 ng/mL 0.5 ng/mL	1.5 ng/mL 1.5 ng/mL	[51]
Nadia Porpiglia et al. (2016)	Hair	LLE	Capillary electrophoresis	R- KET (73) S- KET (89) R- NK (79) S- NK (91)	0.08 ng/mL 0.08 ng/mL 0.08 ng/mL 0.08 ng/mL	0.25 ng/mL 0.25 ng/mL 0.25 ng/mL 0.25 ng/mL	[52]
Donata Favretto et al. (2013)	Hair	LLE	LC–HRMS	KET (88) NK (92)	0.02 ng/ml 0.02 ng/ml	0.05 ng/mL 0.05 ng/mL	[53]
Pai-Sheng Cheng et al. (2007)	Urine	SPE	GC–MS	KET (77.9) NK (73.9) DNK (53.4)	Not Reported	15 ng/mL 10 ng/mL 20 ng/mL	[27]
María Jesús Tabernero et al. (2009)	Hair	LLE	LC–MS/MS	KET (86.56) NK (99)	0.1 ng/mL 0.1 ng/mL	0.5 ng/mL 0.5 ng/mL	[34]
Suling Zhang et al. (2015)	Urine Blood	MSPE	GC–MS	KET (76.56) KET (66.56)	0.24 ng/mL	0.081 ng/mL	[54]
Mark C. Parkin et al. (2008)	Urine	SPE	UHPLC–MS/MS	KET (72) NK (70)	0.03 ng/mL 0.05 ng/mL	0.1 ng/mL 0.1 ng/mL	[30]
N.S. Nosseir et al. (2014)	Blood	LLE	LC–MS/MS	KET (72)	0.01 ng/mL	10 ng/mL	[55]
Magdalena et al. (2021)	Blood	DBS/MAE	Capillary electrophoresis	KET (111)	6 ng/mL	19.8 ng/mL	[17]
Hua-Yang Liao et al. (2015)	Blood	DLLME	GC–MS	KET (111)	20 ng/mL	Not Reported	[16]
Ivo Moreno et al. (2015)	Urine	SPE	GC–MS/MS	KET (100.68) NK (76.68)	5 ng/mL	Not Reported	[56]
Hei Hwa Lee et al. (2015)	Urine	SPE	GC–MS	KET (91.5) NK (89.8)	10 ng/mL 30 ng/mL	25 ng/mL 30 ng/mL	[57]
Cheng et al. (2008)	Urine	SPE	GC–MS	KET (96.5)	15 ng/mL	15 ng/mL	[58]
Harun, N et al. (2009)	Urine	SPE	LC–MS/MS	KET (113.4) KET (102.1)	0.6 ng/mL 0.6 ng/mL	1.9 ng/mL 2.1 ng/mL	[59]
Melent'ev et al. (2004)	Blood	LLE	GC–MS	KET	0.05 ng/mL	Not Reported	[15]
Wendi Zhang et al. (2010)	Urine	……	BA-ELISA	KET (99.9)	0.03 ng/mL	Not Reported	[26]
Huei R. Lin et al. (2010)	Urine	DLLME	GC–MS	KET NK DNK	0.1 ng/mL 0.1 ng/mL 0.1 ng/mL	Not Reported	[20]
Ahai C et al. (2004)	Urine	LLE	GC–MS	KET (97.1) KET (85.7)	0.3 ng/mL 0.15 ng/mL	Not Reported	[31]
Piotr Adamowicz et al. (2004)	Urine	SPE	LC-MS	KET (107) KET (101)	0.5 ng/mL 0. 5 ng/mL	2 ng/mL 2 ng/mL	[60]
Juliana Ribeiro Ibiapina Leitão et al. (2024)	Oral fluid	DLLME	LC-MS-MS	KET	10 ng/mL	10 ng/mL	[32]

List of abbreviations:

KET: Ketamine
NK: Norketamine
HNK: Hydroxynorketamine
DNK: Dehydronorketamine
LOD: Limit of Detection
LOQ: Limit of Quantification
LC-MS: Liquid Chromatography–Mass Spectrometry
LC–MS-MS: Liquid Chromatography/Tandem Mass Spectrometry
LC–HRMS: Liquid Chromatography-High Resolution Mass Spectrometry
DBS-MAE: Dried Blood Spot/Microwave-Assisted Extraction
BAμE: Bar Adsorptive Microextraction and Microliquid Desorption
WT-ESI-MS: Wooden-Tip Electrospray Ionization Mass Spectrometry
MSPE: Magnetic Solid-Phase Microextraction
HF-LPME: Hollow-Fiber Liquid-Phase Microextraction
SFE–SFC–MS: Supercritical-Fluid Chromatography and Single Mass
SFC–UHPLC: Supercritical-Fluid Chromatography and single quadrupole MS detection
MISPE: Molecularly Imprinted Solid-Phase Extraction
CFIA: Competitive Fluorescence Immunoassay
UHPLC-DAD: Ultra-High Performance Liquid Chromatography Coupled With Diode Array Detection
GC-MS-MS: Gas Chromatography–Tandem Mass Spectrometry
GC-M: Gas Chromatography–Mass Spectrometry
FE-GC-MS: Flash Evaporation-Gas Chromatography-Mass Spectrometry
SPE: Solid Phase Extraction
SPME: Solid-Phase Microextraction
BA-ELISA: Biotin–Avidin Amplified Enzyme-Linked Immunosorbent Assay
UHPLC-MS-MS: Ultra-High Performance Liquid Chromatography Tandem Mass Spectrometry
HRMS/MS: High Resolution Mass Spectrometry
DLLME: Dispersive Liquid-Liquid Microextraction
LLE: Liquid-Liquid Extraction
CE-MS: Capillary Electrophoresis–Mass Spectrometry

Urine sample

A urine sample is the most widespread sample because it is easy to sample and harmless to the body. KET and its metabolites can be detected in urine for a certain period after ingestion, depending on factors such as dosage, frequency of use, and individual metabolism. Detection windows typically range from a few days to several weeks. Initial screening tests may be followed by confirmatory tests to ensure the accuracy of results and rule out false positives. In addition to detecting KET itself, testing may also target metabolites such as NK and dehydronorketamine (DNK) for a more comprehensive analysis. KET testing in urine samples may have legal implications, such as workplace drug testing or law enforcement investigations [8, 10, 19, 20]. In studies investigated in this review, 26 were performed on urine samples. For example, HUEI-RU LIN detected KET, NK, and DNK in urine samples by GC-MS without derivatization. In this study, the LOD was 1 ng/mL, 5 ng/mL, and 20 ng/mL for K, NK, and DHNK, respectively. The total analytical time for GC-MS confirmation is 20 min per sample. Moreover, the sensitivity and specificity are 98.9% and 100%, respectively. This screening method is rapid, sensitive, and applicable to forensic and clinical toxicological analyses [21]. Ahmad et al. simultaneously detected KET and NK in urine samples by GC-MS in 0.45 min/sample. In their study, the LOD and LOQ for KET and NK were 1 μg/mL and 5 μg/mL, respectively. The results of this study revealed that the analytical cycle for the quantification of KET and NK in urine samples was much faster, more environmentally friendly, and more cost-effective compared to existing methods [4].

Hair sample

Various pre-treatment approaches have been devised to assess KET in human hair, encompassing washing, digestion, and extraction. Human hair tends to accumulate contaminants, such as dust, sweat, and oils, over prolonged exposure to the environment. Typically, acid digestion is commonly employed due to its gentle conditions, efficient release, minimal background interference, and straightforward operation. Alkaline hydrolysis can fully dissolve hair, ensuring the complete release of analyte molecules, albeit resulting in higher impurity levels in the digestion solution. Enzymatic digestion is rapid, thorough, and specific, but it incurs a higher cost. Organic solvent digestion yields more precise drug forms and concentrations in hair by minimizing analyte hydrolysis during the release process. In recent times, the utility of hair analysis has been demonstrated in forensic and clinical toxicology. In contrast to traditional biological specimens such as blood and urine, hair samples offer a means to chronicle extended drug exposure over time. By segmenting hair strands, it becomes feasible not only to reveal persistent drug consumption but also to infer patterns and durations of drug use based on hair growth rates. Moreover, as a complement to blood and urine specimens, hair samples possess advantageous traits, including their noninvasive nature and resilience against tampering [3, 10, 22]. Of all the studies included in this review, 11 addressed hair samples. In a study by Xiang et al., KET and NK were identified in hair samples using GC–MS. The findings revealed an LOD of 0.02 ng/mL for KET and NK, with an LOQ of 0.05 ng/mL [3]. In another study conducted by Rhee et al., methylenedioxymethamphetamine, methamphetamine, cocaine, propofol, zolpidem, KET, and NK were identified in hair samples using LC–MS/MS with a 1% solution of hydrochloric acid in methanol. For KET and NK, the LOD was reported as 0.001 ng/mL with an LOQ of 0.004 ng/mL. Additionally, it was found that 92.9% of KET abusers were polydrug abusers [5].

Sample Preparation

Various techniques are commonly utilized for KET analysis in biological samples, including liquid-liquid extraction (LLE), solid-phase extraction (SPE), and liquid-phase microextraction (LPME). Solid-phase microextraction (SPME) is a simple and rapid extraction process without the need for solvents. It is suitable for a variety of sample matrices and analytes. Minimizing sample preparation steps, saving time, and reducing the risk of contamination can also be used multiple times for extractions, reducing costs in the long run. Although SPME has limited capacity to extract larger molecules due to the size constraints of the extraction phase, it may not be as sensitive as other extraction techniques. In addition, this technique has limited selectivity compared to other extraction methods. Further, SPME fibers can be fragile and may need careful handling to prevent damage. SPE allows for selective extraction of KET from biological samples, minimizing interference from other compounds. It can enhance the sensitivity of KET detection by concentrating the analyte and help remove matrix components that could interfere with KET analysis, improving the accuracy of the results. SPE methods can be automated, making them efficient for high-throughput analysis in laboratories. Nonetheless, these methods can be costly due to the need for specialized cartridges and equipment. Proper training is necessary for the effective use of SPE techniques. High sample throughput might be limited by the time-consuming nature of some SPE protocols. LLE can be effective for extracting KET from different biological samples. It is often less expensive and relatively simple compared to some other extraction methods. LLE can exhibit lower selectivity in comparison to other extraction techniques. Large volumes of solvents may be required for LLE, leading to increased waste generation. Emulsions can form during LLE, affecting the efficiency of the extraction process. Dispersive liquid-liquid microextraction (DLLME) can offer high extraction efficiency for KET in biological samples. It typically requires minimal amounts of organic solvents, reducing costs and environmental impact. Furthermore, it is a rapid extraction method suitable for high-throughput analysis. DLLME can be more complex compared to traditional extraction methods. DLLME efficiency can be sensitive to experimental parameters, requiring careful control. Emerging methods, such as SPME and DLLME, have become popular due to their efficient extraction of KET from intricate biological matrices [4, 23-25]. This analysis highlights that LLE (16 techniques), SPE (15 techniques), and DLLME (8 techniques) were the most prevalent methods employed for KET sample preparation.

Analytical techniques for detecting ketamine in biological samples

KET in biological samples is typically identified through various laboratory techniques. Immunoassay screening tests are often the initial step to detect the presence of KET in urine samples due to their speed and cost-effectiveness [25-27]. Confirmatory tests, such as GC-MS, LC-MS, and HPLC, provide more accurate and specific results by identifying the exact molecular structure of KET and its metabolites in the urine sample. These methods are crucial for quantifying the concentration of KET present and confirming the initial screening results [19, 28]. Although HPLC has good separation power for KET and related compounds, it may have lower sensitivity compared to MS-based techniques. UHPLC-MS/MS has higher resolution and faster analysis compared to HPLC. It improves sensitivity and selectivity for KET detection. However, it requires skilled operators, complex instrumentation, and higher costs for method development and optimization [29, 30]. For example, Parkin et al. identified KET, NK, and DNK in urine samples using SPE and UPLC–MS/MS. In this study, the detection limit for KET and NK was 0.03 ng/mL and 0.05 ng/mL, respectively, and these compounds could be confirmed in the urine for up to 5 days and 6 days, respectively. DNK was confirmed for up to 10 days, providing a very broad window of detection. The production peak area ratio was observed for KET (m/z 124.8) and NK (m/z 124.9). The retention time (min) for KET, NK, and DNK was 3.59, 3.44, and 2.95, respectively [30]. Flash evaporation-GC-MS (FE-GC-MS) is a technique utilized to analyze volatile or semi-volatile components with low boiling points in samples by evaporation, subsequently separating [10]. For example, Liu et al. detected KET, methamphetamine, and 3,4-methylenedioxymethamphetamine in hair samples using FE-GC-MS. In this study, the LOD and LOQ for KET were reported to be 0.7 ng/mL and 2 ng/mL, respectively. The precision ranged from 1.57% to 7.75% for KET. The results of this study demonstrated that FE-GC-MS was a faster, simpler method for determining KET in human hair compared to traditional time-consuming methods [10]. GC-MS is another widely used technique for analyzing KET in biological samples. It involves separating the components of a mixture using GC and then identifying the compounds using MS [31]. GC-MS has high sensitivity and specificity in detecting KET. It is able to separate and identify compounds in complex mixtures. Although it is a well-established technique with reliable results, it requires sample derivatization for some compounds. In agreement with this finding, Lin et al. identified KET, NK, and DNK in urine samples using GC-MS. They reported the LOD for KET, NK, and DNK as 0.1 ng/mL each, with a total analysis time of 1.5 min/sample [20]. LC-MS is a powerful technique that combines the separation capabilities of LC with the detection and identification abilities of MS. It is highly sensitive and specific, making it ideal for quantifying KET in biological samples. Nonetheless, it requires careful method development and optimization [20, 24, 29]. Consistent with this observation, Oliveira et al. identified KET in oral fluids using LC-MS-MS. The linear range spanned from 10 ng/mL to 1,000 ng/mL, with the LOD and lower LOQ set at 10 ng/mL [32].

DISCUSSION

This systematic review provided a comprehensive analysis of existing methods for the identification of KET and its metabolites in various biological samples. The findings highlight the widespread use of advanced analytical techniques, such as LC-MS and GC-MS, demonstrating high sensitivity and specificity in detecting KET, even at low concentrations. These methods remain the gold standard, yet the need for further refinement continues [19, 29, 33]. One of the key findings of this review was the crucial role of sample type in determining the accuracy and feasibility of KET detection. While blood samples provide information on active drug concentrations, their utility is constrained by a short detection window and vulnerability to postmortem changes [8, 10, 15, 16]. Urine and hair samples offer extended detection periods and non-invasive collection methods, making them valuable alternatives depending on the research or forensic context [8, 10, 19, 20]. Various studies have utilized blood, urine, and hair samples to detect KET [3, 10, 22]. Blood analysis, using techniques such as CE-MS and UHPLC–MS/MS, provides high accuracy with LODs ranging from 0.5 ng/mL to 6 ng/mL [17]. Urine is the most commonly used sample due to easy collection, with GC-MS methods offering high sensitivity and rapid analysis [4]. Hair is useful for detecting long-term KET use, with some methods achieving extremely low LODs down to 0.001 ng/mL [5]. This diversity in biological matrices implies that the selection of sample type should be purpose-driven, balancing factors such as detection window, invasiveness, and logistical feasibility. Another important observation was the impact of sample preparation techniques on analytical outcomes. Methods such as LLE and SPE are widely employed to enhance sensitivity and remove interfering substances [4, 23-25]. However, these techniques often require substantial time and resources, which may not be feasible in routine settings or resource-limited laboratories. This underscores the trade-off between analytical rigor and operational practicality, a consideration that must be tailored to the capabilities of the testing facility. The diversity in analytical protocols and lack of standardization across studies presents a significant limitation in the field. Future research should focus on the development of simplified, cost-effective, and standardized protocols that maintain analytical accuracy while improving accessibility [4, 23-25]. The current review emphasizes this gap by showing that even studies using similar technologies (e.g., LC-MS) often apply varying parameters, extraction techniques, and calibration strategies—making cross-study comparisons challenging. Moreover, emerging technologies, such as ambient ionization MS or portable detection devices, could offer promising alternatives that merit further validation [20, 24, 29]. These tools may be particularly useful in time-sensitive or resource-constrained environments, but their reliability, sensitivity, and specificity compared to conventional methods remain underexplored. In this context, a recent study utilizing advanced UPLC-MS has significantly advanced the field by identifying several novel KET metabolites in pig

brain tissue. These newly detected compounds, such as phenol-hydroxy-NK, DNK, and DHNK, have been structurally confirmed and contribute to a more comprehensive understanding of KET's biotransformation within neural substrates [61]. Such findings not only refine our knowledge of KET metabolism but also open new avenues for improving the interpretive accuracy of toxicological assessments, particularly in neuropharmacological and forensic investigations. This review also underscores the importance of tailoring the analytical approach to the specific goals of the investigation, whether clinical, forensic, or toxicological. For instance, in forensic toxicology, long-term detection in hair may be more relevant, whereas real-time plasma levels might be crucial in the clinical monitoring of KET use or overdose. Our synthesis demonstrated that no single biological matrix or method is universally optimal—highlighting the significance of contextual decision-making in method selection. Overall, while current methods for the identification of KET are reliable, several limitations remain. Addressing these challenges through innovation, standardization, and methodological optimization could significantly improve the accuracy and applicability of KET detection across various biological matrices. Future studies should prioritize the development of rapid, minimally invasive detection methods that are validated specifically for emergency or field settings, such as roadside testing or point-of-care diagnostics.

CONCLUSION

The findings revealed that advanced hyphenated analytical methods combined with MS, such as GC-MS, LC-MS, and related tandem GC-MS and LC-MS, were prominent. Additionally, LLE and SPE were the prevailing methods employed for EKT sample preparation. In general, a comprehensive understanding of the available identification methods for KET and its metabolites is vital for accurate and reliable analyses in a variety of fields, such as forensic investigations, clinical research, and drug monitoring programs.

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